Research Article

Comparative Antidiabetic Profile of Ayurvedic Herbo-mineral Formulation and its Constituents on Normal and Streptozotocin-induced Diabetic Rats

Akansha Mishra, Rohit Srivastava, Arvind K. Srivastava*
Division of Biochemistry, CSIR-Central Drug Research Institute, Lucknow, India.
***Corresponding author's E-mail:** drarv55cdri@rediffmail.com

Accepted on: 06-08-2013; **Finalized on:** 30-09-2013.

ABSTRACT

Madhusunya is a commercially available Ayurvedic polyherbo-mineral preparation composed of nine medicinal plants i.e. *Gymnema sylvestre, Momordica charantia, Syzygium cumini, Azadirachta indica, Withania somnifera, Hibiscus rosa-sinensis, Ocimum sanctum, Pterocarpus marsupium and Coccinia indica* and a herbomineral substance 'Shilajeet'. It improved oral glucose tolerance post sucrose load on both normal as well as STZ-induced diabetic rats, however, the effects of its constituents on oral glucose tolerance was not well marked at the concentration present in the formulation. Madhusunya treatment also resulted in lowering of elevated blood glucose and glycosylated hemoglobin (HbA1c) levels and increasing of declined serum insulin levels of STZ-induced diabetic rats when given for 21 consecutive days. 'Madhusunya' treatment also decreased the elevated levels of triglycerides, total cholesterol, LDL-cholesterol and increased the declined HDL-cholesterol levels in sera of STZ-induced diabetic rats. The renal as well as hepatic pathology were also found improved in the STZ-induced diabetic rats treated with Madhusunya. However, the effect of constituents except on hepatic parameters and renal function parameters was not prominent. This study provides at least some logic for using antidiabetic polyherbomineral formulation rather than the individual plant powder in the treatment of dysglycemia and associated complications in T2DM patients in the ayurvedic system of medicine.

Keywords: Antihyperglycemic activity, Diabetes mellitus, Diabetic complications, Madhusunya.

INTRODUCTION

Non insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes mellitus (T2DM) is a commonest endocrine disorder affecting nearly 6.0 % of the population worldwide. It is caused either by decreased production of insulin from pancreas or its improper action, both results in increase of blood glucose levels in the blood stream. Persistent hyperglycemia in the blood stream is one of the causative factors for damaging many of the body systems, particularly the blood vessels and nerves.[1,2] Ayurveda, the traditional Indian herbal medicinal system practiced over thousands of years have reports of herbal antidiabetic formulations with no known side effects. However, these formulations have not gained much importance as medicines and one of the factors is lack of specific standards being prescribed for herbal medicines and supportive animal/clinical trials.[3] However, many of the constituent plants have been widely prescribed for diabetic treatment all around the world with less known mechanistic basis of their functioning. Madhusunya is a commercially available poly herbo-mineral formulation composed of nine medicinal plants i.e. *Gymnema sylvestre, Momordica charantia, Syzygium cumini, Azadirachta indica, Withania somnifera, Hibiscus rosa-sinensis, Ocimum sanctum, Pterocarpus marsupium and Coccinia indica* and a herbo-mineral substance 'Shilajeet'. Madhusunya has been recommended for use in the management of hyperglycemia in T2DM patients.

Gymnema sylvestre, a member of the milkweed family Asclepiadaceae is a woody plant found in the tropical forest of India and Africa. The medicinally active parts have been reported leaves and roots although the exact mechanism of antidiabetic action of the plant is still not known. Besides impairing the ability to discriminate sweet taste, increase enzyme activity responsible for the glucose uptake and utilization, it may stimulate pancreatic beta-cell function, increase beta-cell number and increase insulin release by increasing cell permeability to insulin.[4] The fruits, leaves and roots of Momordica charantia (Family: have been shown to exhibit various biological activities including antidiabetic, anti rheumatic, antiulcer, anti-inflammatory and antitumor, and is used for treating jaundice, leprosy and as anti venom to snakebite.[5] Although the seeds of the plant Syzygium cumini (Family Myrtaceae) have been established for its antidiabetic potential in ayurveda as well as modern scientific community, S.cumini seeds have been reported for hypoglycemic, anti-inflammatory, neuropsycho-pharmacological, anti-bacterial, anti-HIV, and anti-diarrheal effect.[6] *Azadirachta indica* (Neem) is perhaps the most useful traditional medicinal plant in India. It has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. Every part of the neem tree has some medicinal property and is thus commercially exploitable.[7] *Withania somnifera* (family: Solanaceae), is widely used in ayurvedic system of medicine in India. It is an official drug and is mentioned in the Indian Pharmacopoeia.[8] Several studies on this plant indicated that it possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic and rejuvenating properties beside its positive influence on the endocrine, cardiopulmonary and central nervous system.[9] An ayurvedic herbal formulation 'Tarsina' containing this

plant as one of the ingredients have been found to attenuate STZ-induced hyperglycemia and pancreatic islet superoxide dismutase activity in type 1 diabetic rats.[10] Hibiscus rosa-sinensis is another ayurvedic remedy that has been mentioned in many ancient Indian medical literatures and is reported to possess antipyretic, anticomplementary, anti-diarrheic, hypolipidemic and antihyperglycemic properties.[11] *Pterocarpus marsupium* Roxb. (Family: Leguminoseae) is advocated for the treatment of diabetes mellitus in Indian system of medicine. Sometimes, unpublished claims have been made that the crude powder of the heart wood of this plant is capable of weaning the patients away from the conventional antidiabetic drugs, which, however, is not true for the presently established antidiabetic drugs.[12] *Coccinia indica* which is grown abundantly in India have been widely used in the traditional treatment of diabetes mellitus. Prasanakumar et al reported hypoglycemic action of the pectin isolated from the fruits of Coccinia indica.[13] Shilajit is a herbo-mineral drug which oozes out from a special type of mountain rocks in the peak summer months. The active constituents of shilajit consist of dibenzo-alpha-pyrones and related metabolites, small peptides (constituting non protein amino acids), some lipids and carrier molecules (fulvic acids). Shilajit finds extensive use in Ayurveda for diverse clinical conditions. For centuries people living in Himalaya and adjoining regions have used shilajit alone or in combination with other plant remedies to prevent and combat problems with diabetes.[14] Studies done by Gupta[15] and Bhattacharya[16] have also reported the antidiabetic action of Shilajit.

There are no doubts that plants as well as Shilajit are wonderful pharmacies and it is also not surprising that a single plant has various activities and combination of various plants may have better in controlling hyperglycemia than either alone as NIDDM or T2DM is a multi-factorial disease. There are scanty reports that the combinations of extracts of different plants exert beneficial effects on diabetic animals. Studies are extremely important to investigate the effects of polyherbo-mineral formulations on blood sugar and other biochemical parameters on diabetic animals. The present study was therefore undertaken to evaluate for the comparative antihyperglycemic effects of a polyherbal formulation "Madhusunya" and the constituents of Madhusunya on validated animal models in the first instance.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma–Aldrich Chemicals Co., St Louis, MO, USA. The biochemical assays for the measurement of total triglycerides (TG), total Cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate amino transferase (AST), alanine amino transferase (ALT), total bilrubin, urea, uric acid and creatinine were purchased from Dialab, India. The kit for the measurement of insulin in the serum was purchased from Mercodia, Uppasala, Sweden**.** Glucostrips used for the estimation of blood glucose levels were purchased from Roche (India). The crude powder of the parts of the constituent plants *Gymnema sylvestre*, *Momordica charantia*, *Syzygium cumini*, *Azadirachta indica*, *Withania somnifera, Hibiscus rosa-sinensis*, *Ocimum sanctum*, *Pterocarpus marsupium*, *Coccinia indica* were purchased from Tansukh, India. The Shilajeet and polyherbo-mineral formulation Madhusunya were the products of ayurvedic medicines manufacturers Vaidhyanath and Meghdoot, India, respectively.

Animals

Male Sprague-Dawley strain of albino rats weighing around 160±20 g used in the present study were procured from National Laboratory Animal Centre, of the Institute. Animals were fed *ad Lib* the pellet diet and water. The following conditions were always maintained in the animal room temperature 24-28 ℃, relative humidity 60-70%, air changes 6 to 10 per hour and 12h day and night cycle. All the experiment on animals was carried out according to IAEC guidelines.

Experimental protocol

Assessment for improvement on glucose tolerance in normal rats

Prior to use, the animals were deprived of food for 16 hrs. Rats showing fasting blood glucose between 60-80 mg/dl were finally selected and randomly divided into groups consisting of five to six animals in each. One group served as control received an equal amount of 1.0% gum acacia used for suspending the test samples. The other groups received either the suspension of the crude powder of the constituent plants or Shilajeet or poly herbo-mineral formulation at the preselected doses. The animals of all the groups were given sucrose (10.0 gm/kg orally) exactly 30 min post administration of the test samples. The blood glucose level of each animal was again measured at 0, 30, 60, 90 and 120 min post sucrose load. Food but not water was withheld from the cages during the course of experimentation. A graph was plotted the blood glucose level and time on y and x axis respectively and area under curve of each group determined. Comparing the AUC of experimental and control group determined the percent improvement on oral glucose tolerance (OGTT).

Assessment of antidiabetic effect by measuring fall in blood glucose level on streptozotocin-induced diabetic rats

Single dose effect

Fasting blood glucose level of each animal was determined after an overnight fast with free access to water. On the following day, streptozotocin was administered at a dose of 60 mg/kg intraperitoneally using a 5.0 % solution of freshly prepared streptozotocin in 0.1 M citrate buffer (pH 4.5). Fasting blood glucose was

measured 2 days after administration of STZ. The animals showing blood glucose level >270 mg/dl were separated, divided randomly into groups (n=6 per group). One group served as control (untreated rats) given 1% Gum acacia whereas the other groups received the suspension of the test samples either the crude powders of the constituent plants or shilajit or poly herbo-mineral formulation at the preselected doses. The blood glucose level of each animal was again measured at 0, 30, 60, 90, 120, 180, 240, and 300 min, post test sample administration. Food but not water was withheld from the cages during the course of experimentation. The blood glucose level of each animal was again measured at 1440 min. A graph was plotted between the average blood glucose level of each group and time on y and x axis respectively and AUC determined. The % fall on blood glucose values from 0 to 300min and 0-1440min by test samples was calculated by comparing the AUC of experimental and control groups.

Multiple dose effects

The induction of diabetes, dividing the diabetic animals into groups and treatment with test samples were the same as described above. The treatment with test samples i.e. suspension of the crude powders of the constituent plants, shilajit or poly herbo-mineral formulation at the pre selected doses was continued for 21 days (one dose per day). At the end of the experiment, the blood of each animal was collected from retro orbital plexus. Serum separated and used for the estimation of total triglycerides (TG), total Cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and aspartate amino transferase (AST), alanine amino transferase (ALT) activities, total bilrubin, urea, uric acid and creatinine on semi auto analyzer (IRIS) using the assay kits according to instructions of the manufacturer. Glycosylated haemoglobin (HbA1c) was measured in the whole blood by HbA1x assay kit as supplied by Dialab, India. Serum insulin levels were measured using an enzyme-linked immunosorbant assay kit (Mercodia, Uppsala, Sweden).

Statistical analysis

Statistical comparison between the control and experimental groups was made by Student's "t" test. Results were expressed as mean ± S.E.M. The results were considered statistically significant, more significant and most significant if the p values were observed 0.05, 0.01 and 0.001, respectively.

RESULTS

Table 1a presents the effect of single dose of the crude powder of the individual constituents of Madhusunya i.e. *G. sylvestre, M. charantia, S. cumini, A. indica, W. somnifera, H. r. sinensis, O. sanctum, P. marsupium, C. indica,* shilajeet and Madhusunya on the improvement of OGTT in normal rats as well as streptozotocin (STZ)-induced diabetic rats post sucrose load. It is evident from the results that the poly herbo-mineral formulation madhusunya cause significant improvement in OGTT of normal as well as STZ-induced diabetic rats at 250 mg/kg dose whereas among the constituents only the crude powder of *G. sylvestre* leaves and *M. charantia* fruits showed improvement in OGTT of the normal rats to the tune of 14.8 and 10.4 %, respectively at the respective doses present in the formulation i.e. 37.5 and 25.0 mg/kg. The crude powder of other constituent plants and shilajeet did not produce any significant improvement on OGTT in the normal rats at the doses present in the formulation. Neither of any of the constituents Madhusunya caused any significant effect on the improvement of OGTT of STZ-induced diabetic rats at the doses present in the formulation.

Table 1b presents the effect of the crude powder of the constituents of madhusunya and madhusunya on the improvement of OGTT of STZ-induced diabetic rats at equal doses. The results obtained present definite evidence that all the constituents have the capability of improving OGTT of both normal as well as STZ-induced but at higher doses i.e. 250 mg/kg, however, the effect of madhusunya on OGTT of both normal and STZ-induced diabetic rats was approximately 1.5 to 3.0 fold higher than the individual constituents at this dose.

Table 1a: Effect of Madhusunya and its constituents on improvement of oral glucose tolerance post sucrose load in normal rats.

Test Sample	Dose (mg)	% improvement on OGTT of normal rats 0-120 min
Gymnema sylvestre leaves	37.5	14.8*
Momordica charantia fruits	25.0	10.4*
Syzygium cumini seeds	37.5	7.39
Azadirachta indica bark	25.0	5.72
Withania somnifera bark	25.0	9.86
Hibiscus rosa-sinensis flowers	12.5	8.84
Ocimum sanctum rhizome	22.5	Nil
Pterocarpus marsupium wood	37.5	Nil
Coccinia indica fruits	25.0	3.69
Shilajeet powder	2.50	6.01
Madhusunya powder	250	30.6**

Number of animal in each group were kept six
Statistical significance *p<0.05, **p<0.01, ***p<0.001

Table 2a present the effect of crude plant powders of the individual constituents of madhusunya and madhusunya itself on blood glucose lowering activity profile on STZ-induced diabetic rats. The crude powder of the

constituent plants at the dose these were mixed in formulation and at 250 mg/kg dose showed little decline while in the same concentration in formulation showed significant decline in blood glucose level. The antihyperglycemic activity of formulation were calculated to around 25.2 (p<0.01) at 0-5h and 28.4 (p<0.01) at 0-24h.

Table 2b shows the effect of crude powder of plant parts and polyherbal formulation madhusunya. Crude powder of plant parts at 250 mg/kg dose produced mild lowering in the blood glucose level while the formulation at the same dose level produced marked lowering in the blood glucose level. The antihyperglycemic activity of formulation were calculated to around 28.9 (p<0.01) at 0-5h and 39.5 (p<0.001) at 0-24h.

Table 3 presents the multiple dose effect of madhusunya and its constituents i.e., *G. sylvestre* leaves, *M. charantia* fruits, *S. cumini* seeds, and *P. marsupium* wood, *A. indica* bark, *W. somnifera* bark, *C. indica* fruits, *H. r. sinensis* flowers and *O. sanctum* root and shilajeet on fasting blood glucose, glycosylated hemoglobin (HbA1c) and serum insulin profile of streptozotocin induced diabetic rats. It is evident from the results that madhusunya and its constituents when given to streptozotocin-induced diabetic rats at 100 mg/kg dose for 21 days caused decline in their fasting blood glucose profile and HbA1c levels. The effect was more pronounced with formulation compared to the effect of individual constituent. The respective decline in fasting blood glucose and HbA1c levels were calculated to be around 48.1 % (p<0.01) and 34.6 % (p<0.01) by madhusunya and 29.8 % (p<0.05) and 27.8 % (p<0.01) by *G. sylvestre* leaves and 35.4 % (p<0.01) and 26.8 % (p<0.01) by *P. marsupium* heartwood, 20.7 % (NS) and 11.2 % (NS) by *A. indica* bark, 24.6 % (p<0.05) and 16.9 % (NS) by *W. somnifera* bark, 26.8 % (p<0.05) and 26.8 % (NS) by *C. indica* fruits, 21.3 % (NS) and 11.2 % (NS) by *H. r. sinensis* flowers and 17.4 % (NS) and 12.3 % (NS) by *O. sanctum* root and 36.1 % (p<0.01) and 21.9 % (NS) by shilajeet. However Madhusunya and its constituents also caused increase in serum insulin level but only Madhusunya, *P. marsupium* wood, shilajeet caused significant increase in serum insulin level by 59.3 % (p<0.01), 43.6 % (p<0.05), 35.6 % (p<0.05) respectively on day 21st whereas other plants i.e., *G. sylvestre* leaves, *M. charantia* fruits, *S. cumini* seeds, *A. indica* bark, *W. somnifera* bark, *C. indica* fruits, *H. r. sinensis* flowers and *O. sanctum* root caused increase in serum insulin levels non-significantly by 27.6 %, 10.9 %, 22.3 %, 16.2 %, 12.5 %, 19.0 %, 10.3 % and 13.5 % respectively.

Table 1b: Effect of Madhusunya and its constituents on oral glucose tolerance post sucrose load on normal and streptozotocin-induced diabetic rats.

Test Sample	Dose (mg)	% Improvement		
		Normal rats	Sucrose challenged STZ- induced diabetic rats	
		0-120min	0 - 300min	0-1440min
G. sylvestre leaves	250	21.2**	18.2**	24.3**
M. charantia fruits	250	13.0*	18.6**	23.3**
S. cumini seeds	250	20.1**	8.20	19.7**
A. indica bark	250	10.9*	24.9**	24.6**
W. somnifera bark	250	19.5**	27.2**	20.8**
H. rosa-sinensis flowers	250	9.72	12.7*	15.5*
O. sanctum rhizome	250	10.6	6.41	12.8*
P. marsupium wood	250	24.6**	22.2**	26.4**
C. indica fruits	250	20.8**	29.3**	26.0**
Shilajeet powder	250	17.7**	14.3*	18.6**
Madhusunya powder	250	30.7**	30.3**	39.6***

Number of animal in each group were kept six; Statistical significance *p<0.05, **p<0.01, ***p<0.001

Table 4 shows the effect of Madhusunya and its constituents i.e., *G. sylvestre* leaves, *M. charantia* fruits, *S. cumini* seeds, and *P. marsupium* wood, *A. indica* bark, *W. somnifera* bark, *C. indica* fruits, Shilajeet, *H. r. sinensis* flowers and *O. sanctum* root on serum lipid profile of streptozotocin induced diabetic rats. It is evident from the data obtained that madhusunya and its constituents when fed to streptozotocin-induced diabetic rats at 100 mg/kg dose caused decline in their serum triglyceride, total cholesterol, LDL-cholesterol and increase HDL-cholesterol levels. Treatment of madhusunya for 21 days showed significant lowering in triglyceride, total cholesterol and LDL-cholesterol content by around 30.8 (p<0.01), 26.4 (p<0.01) and 29.3 % (p<0.01) and increase in HDL-cholesterol content by around 26.4 % (P<0.05) when given at 100 mg/kg dose in diabetic rats.

Table 5 shows the effect of madhusunya and its constituents i.e., *G. sylvestre* leaves, *M. charantia* fruits, *S. cumini* seeds, and *P. marsupium* wood, *A. indica* bark, *W. somnifera* bark, *C. indica* fruits, Shilajeet, *H. r. sinensis* flowers and *O. sanctum* root on serum renal and hepatic profile of streptozotocin induced diabetic rats. It was evident from table 10 that the treatment of crude powder of constituent plants showed reduction in serum urea,

uric acid and creatinine. Standard formulation madhusunya showed marked reduction in urea, uric acid and creatinine by around 43.7 (P<0.01), 39.6 (P<0.01) and 42.8 % (P<0.01). little reduction in GOT, GPT and total bilrubin was seen post treatment of crude plant powders while madhusunya showed mark reduction in serum GOT, GPT and total bilrubin in these rats which were calculated to around 33.4 (P<0.01), 34.4 (P<0.01) and 45.7 % (NS) post treatment.

Table 2a: Blood glucose lowering effect of Madhusunya and its constituents on streptozotocin-induced diabetic rats

Test Sample	Dose (mg)	% Antihyperglycemic activity	
		0 min - 300 min AUC	0 min - 1440 min AUC
G. sylvestre leaves	37.5	10.1	6.65
M. charantia fruits	25.0	9.98	10.4
S. cumini seeds	37.5	7.75	11.8
A. indica bark	25.0	6.28	5.37
W. somnifera bark	25.0	7.38	10.6
H. rosa-sinensis flowers	12.5	7.86	10.6
O. sanctum rhizome	22.5	13.9	11.9
P. marsupium wood	37.5	6.05	3.45
C. indica fruits	25.0	13.1	16.5
Shilajeet powder	2.50	11.2	13.6
Madhusunya powder	250	25.2**	28.4**

Number of animal in each group were kept six; Statistical significance *p<0.05, **p<0.01, ***p<0.001

Table 2b: Blood glucose lowering effect of madhusunya and its constituents on streptozotocin-induced diabetic rats

Test Sample	Dose (mg)	% Antihyperglycaemic activity	
		0 min - 300 min AUC	0 min - 1440 min AUC
G. sylvestre leaves	250	15.1*	26.4**
M. charantia fruits	250	16.2	27.7**
S. cumini seeds	250	11.0	24.9**
A. indica bark	250	22.8*	23.9**
W. somnifera bark	250	17.7*	21.4**
H. rosa-sinensis flowers	250	9.27	22.4**
O. sanctum rhizome	250	6.85	14.3
P. marsupium wood	250	24.6**	27.2**
C. indica fruits	250	17.6*	22.4**
Shilajeet powder	250	14.2*	21.1**
Madhusunya powder	250	28.9**	39.5***

Number of animal in each group were kept six; Statistical significance *p<0.05, **p<0.01, ***p<0.001

Table 3: Effect of Madhusunya and its constituents on fasting blood Glucose (FBG), glycosylated hemoglobin (HbA1c) and serum insulin of streptozotocin-induced diabetic rats

Groups	FBG[1] (mg/dl)	HbA1c[1] (mg/dl)	Insulin[1] (µg/l)
Control	251±8.91	8.15±0.29	0.29±0.01
G. sylvestre[2]	176±15.2** **-29.8**	5.92±0.31 **-27.8**	0.38±0.08 **+27.6**
M. charantia[2]	212±22.5 **-15.4**	6.86±0.62 **-15.7**	0.33±0.04 **+10.9**
S. cumini[2]	194±21.8* **-22.8**	6.44±0.59 **-20.9**	0.36±0.02 **+22.3**
A. indica[2]	199±14.9 **-20.7**	7.24±0.68 **-11.2**	0.34±0.004 **+16.2**
W. somnifera[2]	189±8.97* **-24.6**	6.77±0.48 **-16.9**	0.34±0.03 **+12.5**
Hibiscus rosa-sinensis[2]	198±5.82 **-21.3**	7.23±0.39 **-11.2**	0.33±0.01 **+10.3**
O. sanctum[2]	207±9.51 **-17.4**	7.14±0.53 **-12.3**	0.34±0.008 **+13.5**
Pterocarpus marsupium[2]	162±8.21** **-35.4**	5.96±0.20 **-26.8**	0.43±0.06 **+43.6**
Coccinia indica[2]	184±8.01* **-26.8**	6.72±0.34 **-26.8**	0.35±0.05 **+19.0**
Shilajeet[2]	160±15.6** **-36.1**	6.36±0.4 **-21.9**	0.40±0.02 **+35.6**
Madhusunya[2]	130±11.3** **-48.1**	5.32±0.06* **-34.6**	0.47±0.04* **+59.3**

[1]on day 21st, [2]- 100 mg/kg dose; Values are mean±SE of six rats. Data in parenthesis is the % change as compare to its control, (-) sign denotes decrease in activity and (+) sigh denotes increase in activity; Statistical significance *p<0.05, **p<0.01, ***p<0.001.

Table 4: Effect of Madhusunya and its constituents on serum lipid profile of streptozotocin-induced diabetic rats

Groups	Lipid profiles			
	TG (mg/dl)	Chol (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	133.9±7.01	89.1±1.62	75.9±1.84	32.0±0.55
G. sylvestre	113.6±4.97 **-15.2**	81.4±4.55 **-8.61**	75.2±4.87 **-0.96**	34.8±1.25 **+8.56**
M. charantia	120.8±12.6 **-9.78**	83.4±3.74 **-6.39**	71.4±6.82 **-6.00**	32.9±1.20 **+2.72**
S. cumini	122.7±9.69 **-8.36**	88.6±1.58 **-0.53**	72.4±4.06 **-4.73**	32.4±1.4 **+1.23**
A. indica	106.2±2.18 **-20.7**	77.7±5.32 **-12.8**	69.1±5.29 **-8.97**	33.4±2.35 **+4.43**
W. somnifera	100.4±3.58* **-25.0**	76.7±5.49 **-13.9**	64.8±4.41 **-14.6**	34.3±3 **+7.25**
H. r. sinensis	134.4±22.9 **-0.37**	78.4±4.91 **-11.9**	69.8±6.55 **-8.03**	35.3±3.37 **+10.1**
O. sanctum	128.3±9.16 **-4.18**	74.6±2.71 **-16.3**	72.2±4.26 **-4.91**	31.8±4.45 **+0.78**
P. marsupium	116.2±7.62 **-13.2**	82.3±5.23 **-7.68**	72.7±5.89 **-4.25**	33.4±1.38 **+4.40**
C. indica	112.7±2.31 **-15.8**	75.1±1.89 **-15.7**	71.0±2.42 **-6.49**	33.1±3.91 **+3.49**
Shilajeet	103.3±2.35 **-22.8**	77.9±4.44 **-12.6**	69.4±3.21 **-8.68**	34.3±3.67 **+7.09**
Madhusunya	92.7±2.26** **-30.8**	65.6±8.39** **-26.4**	53.7±0.93** **-29.3**	40.5±1.26* **+26.4**

[1]on day 21[st], [2]- 100 mg/kg dose; Values are mean±SE of six rats. Data in parenthesis is the % change as compare to its control; (-) sign denotes decrease in activity and (+) sign denotes increase in activity; Statistical significance *p<0.05, **p<0.01, ***p<0.001.

Table 5: Effect of madhusunya and its constituents on renal and liver function profile of streptozotocin-induced diabetic rat

Groups	Renal function profiles			Liver function profiles		
	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	GOT (U/l)	GPT (U/l)	T-Bilrubin (mg/dl)
Control	151.5 ±8.62	5.24 ±0.23	0.64 ±0.04	178.5±22.5	165.3±8.97	0.35±0.02
G. sylvestre	135.3±12.1 **-10.7**	4.23±0.36 **-19.3**	0.50±0.05 **-21.4**	145.8±7.14 **-18.3**	137.4±7.95 **-16.9**	0.29±0.02 **-17.1**
M. charantia	129.0±5.95 **-14.8**	4.57±0.52 **-12.8**	0.48±0.04* **-25.3**	144.0±5.97 **-19.3**	130.0±13.8* **-21.4**	0.28±0.17 **-20.0**
S. cumini	140.2±8.89 **-7.46**	3.93±0.25 **-25.0**	0.52±0.03 **-18.9**	147.2±7.12 **-17.5**	139.7±8.69 **-15.5**	0.26±0.07 **-25.7**
A. indica	119.9±5.48* **-20.8**	4.44±0.42 **-15.4**	0.46±0.04** **-28.3**	163.6±11.1 **-8.35**	141.2±9.16 **-14.6**	0.33±0.04 **-5.71**
W. somnifera	123.4±4.82 **-18.5**	3.84±0.46 **-26.7**	0.45±0.05** **-30.2**	151.8±11.8 **-14.9**	144.0±7.49 **-12.9**	0.32±0.06 **-8.57**
H. r. sinensis	132.5±3.26 **-12.5**	4.47±0.46 **-14.7**	0.51±0.03 **-20.1**	162.3±8.57 **-9.08**	148.0±11.9 **-10.5**	0.34±0.04 **-2.86**
O. sanctum	139.6±13.7 **-7.85**	5.08±0.62 **-3.05**	0.52±0.03 **-18.8**	164.8±7.54 **-7.67**	152.5±10.2 **-7.74**	0.32±0.03 **-8.57**
P. marsupium	120.2±9.55* **-20.7**	4.23±0.50 **-19.3**	0.46±0.03** **-28.8**	138.2±7.36 **-22.6**	122.4±6.59** **-25.9**	0.30±0.05 **-14.3**
C. indica	130.7±2.96 **-13.7**	4.32±0.52 **-17.5**	0.48±0.04* **-24.9**	161.3±10.8 **-9.64**	149.7±5.74 **- 9.44**	0.31±0.03 **-11.4**
Shilajeet	107.5±4.28** **-29.0**	3.43±0.27* **-34.6**	0.42±0.04** **-34.5**	139.5±13.9 **-21.8**	126.3±2.94* **-23.6**	0.27±0.04 **-22.8**
Madhusunya	85.3±5.25** **-43.7**	3.16±0.33** **-39.6**	0.37±0.02** **-42.8**	118.8±13.3** **-33.4**	108.4±2.89** **-34.4**	0.19±0.03 **-45.7**

[1]- on day 21[st], [2]- 100 mg/kg dose; Values are mean±SE of six rats. Data in parenthesis is the % change as compare to its control.
(-) sign denotes decrease in activity and (+) sigh denotes increase in activity; Statistical significance *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

During diabetes mellitus the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels.[6] Streptozotocin (STZ), a β-cytotoxin, induces 'chemical diabetes' in a wide variety of animal species including rats by selectively damaging the insulin-secreting β-cells of the pancreas. Intraperitoneal injection of STZ produces fragmentation of DNA of β-cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of β-cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinemia.[17, 18] Tahiliani et al., [19] also reported STZ-diabetes in type 2 diabetics produce a significant increase in glucose levels associated with decrease in insulin levels. The serum glucose, lipid and cholesterol values as observed for the rats in the present study are in agreement with those expected for streptozotocin diabetic rats.[20, 21]

Since from ancient time, plants and herbal formulation are used in the treatment of diabetes mellitus but there is a little report regarding the scientific validation and efficacy of the antidiabetic activity and the rationale of using polyherbal formulation. The primary objective of the study was to examine the antidiabetic potential of repeated administration of individual crude plant parts and standard formulation madhusunya for 21 days in STZ-induced type 2 diabetic rat model in term of significant antihyperglycemic activity and to see the rational of using polyherbal formulation instead of single one.

Gymnema sylvestre is a plant used in India and parts of Asia as a natural treatment for diabetes.[22] The antihyperglycemic activity of crude powder of *Gymnema sylvestre* leaves was confirmed in the multiple dose experiments on streptozotocin-induced diabetic rats. The increased levels of blood glucose in STZ-induced diabetic rats were found lowered by the administration of *Gymnema sylvestre* crude leaf powder. Fasting blood glucose was improved with the continuous treatment with the plant powder. The similar anti-diabetogenic effects were reported by Kanetkar et al.[23] The reduced glucose levels suggested that crude extract might exert through the insulin release by the stimulation of a regeneration process and revitalization of the remaining beta cells.[24,25] This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with crude powder of plant leaves which may stimulates insulin secretion from regenerated β cells or caused the release of insulin from the residual β cells. Increased serum insulin level in *Gymnema sylvestre* administered diabetic rats suggests the insulinotropic activity of *Gymnema sylvestre* leaf extract. Liu et al, [26] also demonstrated the effect of *Gymnema sylvestre* aqueous leaf extract on insulin secretion. Crude leaf powder of *Gymnema sylvestre* also improved the glycosylated haemoglobin and increase in serum insulin levels. The marked increase in serum triglycerides, total cholesterol, LDL-cholesterol and decreased HDL-cholesterol observed in diabetic rats is in agreement with the findings of Nikkila and Kekki.[27] In the present study, administration of the crude powder of leaves to the STZ-induced diabetic rats significantly improved these parameters. Serum urea, uric acid and creatinine levels also increased in STZ-induced diabetic rats.[28] Crude leaf powder of *Gymnema sylvestre* also improves the renal function tests. Streptozotocin treatment has a significant role in the alteration of liver functions since the activity of AST, ALT and T-bilrubin was significantly higher than those of normal value. On the other hand, treatment of the diabetic rats with *Gymnema sylvestre* leaves powder caused reduction in the activity of these enzymes in plasma compared to the mean values of the diabetic group.

Momordica charantia, a very common medicinal plant used in the treatment of hyperglycemia and dyslipidemia.[29] Glucose lowering activity of crude powder of fruit of plant was confirmed in multiple dose experiment on streptozotocin-induced diabetic rats. *M. charantia* showed reduction in fasting blood glucose concentration and significant improvement in the response to an oral glucose load. Leatherdale et al[30] also reported the similar study. There is mild increase in the insulin level post 21 days treatment in the diabetic rats. A mild reduction in glycosylated haemoglobin was noticed in *M. charantia* treated group. There is slightly decrease in serum triglyceride, total cholesterol, LDL-cholesterol and mild increase in HDL-cholesterol. Serum urea, uric acid and creatinine levels significantly decrease with fruit treatment. This showed that crude powder of fruit of *Momordica charantia* may help in kidney damage in diabetes mellitus. Treatment of the diabetic rats with *Momordica charantia* fruit powder also caused reduction in the activity of AST, ALT and T-bilrubin.

Ayurveda prescribed *Syzygium cumini* as an ancient Indian form of medicine.[31] The seeds of this plant have been reported to possess many medicinal properties in the Ayurveda system of medicine.[6] After the chronic administration of crude powder of *Syzygium cumini* seeds for 21 days caused significant reduction in blood glucose levels. These results confirmed the use of *Syzygium cumini* seed of traditional practice as an antidiabetic.[32] The possible mechanism by which seed brings about a decrease in blood sugar levels may be potentiation of the insulin effects of plasma by increasing either the pancreatic secretion of insulin from beta cells of the islets of Langerhans or its release from the bound form. [33] There was no significant reduction in the fasting blood glucose, glycosylated haemoglobin. Little increase in the serum insulin levels was noticed in *S. cumini* treated group. No significant reduction was observed in the serum triglyceride, total cholesterol, LDL-cholesterol and mild increase in HDL-cholesterol levels. Treatment of seeds of the plant showed little reduction in serum urea and significant reduction in uric acid and T-bilrubin. Crude powder of seeds has little effect on liver function test in diabetic rats.

Azadirachta indica is an indigenous tree grown all over India and is attributed to have many medicinal properties. *Azadirachta indica* is known to possess hypolipidemic, hypoglycemic, immunostimulant and hepatoprotective properties, while nimbocinone, nimolinone, kulactone, nimocinolides, isonimocinolide, nimbin, salanin, azadirachtin, flavonoids, myricetin, meldenindiol, vilasinin, margosinolide, isomargosinolide, desacetyl dihydronimbic acid have been isolated from *A. indica* leaves having medicinal properties.[34] The continuous treatment of the crude powder of bark of *Azadirachta indica* for the period of 21 days produced a significant tolerance in the blood sugar levels of diabetic rats. Antihyperglycemic/hypoglycemic activity of neem leaves in dogs and neem seed oil was reported.[35] The exact mechanism in reducing the blood glucose level is not well clear but the probable cause of reduction of blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor.[36] Treatment with crude bark of plant showed

mild lowering in the fasting blood glucose, glycosylated haemoglobin and mild increase serum insulin levels. Treatment with crude bark of plant significantly lowered the serum triglyceride levels and no significant lowering was recorded in total cholesterol and LDL-cholesterol. There was very little increase in HDL-cholesterol level. Bark of the plant significantly restored the kidney function profile. There was significant lowering in ALT, AST and T-bilrubin noticed after chronic treatment of bark of *Azadirachta indica*.

Withania somnifera is an important medicinal plant, which is used in traditional medicine to cure many diseases. [37] Administration of crude bark of *Withania somnifera* for 21 days to diabetic rats decreased the level of blood glucose significantly. Hypoglycemic effects and effects of *Withania somnifera* on insulin sensitivity in non-insulin dependent diabetic rats have been reported. [9] A fall on fasting blood glucose and glycosylated haemoglobin and little increase in serum insulin levels were noticed in *Withania somnifera* treated group. There was a significant fall on serum triglyceride but no promising effect on total cholesterol, LDL-cholesterol and HDL-cholesterol. Crude bark of *Withania somnifera* plant showed serum renal and liver protective activity post treatment on streptozotocin-induced diabetic rats.

Hibiscus rosa-sinensis has been used for the treatment of a variety of diseases as well as to promote wound healing.[38] Repeated oral administration of crude powder *Hibiscus rosa-sinensis* flower for 21 days showed significant glucose tolerance in streptozotocin-induced diabetic rats. Similar findings on leaf extract of *Hibiscus rosa-sinensis* have been documented.[39] No promising result was obtained with treatment of plant part as administration of plant part slightly lowered the fasting glucose level, glycosylated haemoglobin and mild increase in serum insulin levels. Hyperlipidemia was observed in diabetic rats. Slightly lowering the triglyceride, total cholesterol and LDL-cholesterol levels and mild increase in HDL-cholesterol level was observed. Severe diabetes may interfere with the normal function of the kidney. This observation is further strengthened by the increased urea, uric acid and creatinine concentration in diabetic rats, oral administered of the crude powder of flower of plant for 14 day lowered the urea, uric acid and creatinine levels. In addition, AST, ALT and T-bilrubin concentrations were also increased in these rats compared with control. Administered with the crude extract showed reduction in AST, ALT and T-bilrubin levels compared with diabetic control. Similar finding were observed by Kate et al. [40]

In Ayurveda Tulsi (*Ocimum sanctum* L.) has been well documented for its therapeutic potentials and described as Dashemani Shwasaharni (antiasthamatic) and antikaphic drugs (Kaphaghna). [41] Leaves of this plant have been used in traditional remedies to control diabetes since antiquity. Anabolic, hypotensive, smooth muscle relaxant and anti stress activity have also been reported.[42] Continuous treatment of crude powder of root of the plant for 21 days was lowered the fasting blood glucose level and significantly improves the glucose tolerance. Antihyperglycemic and antihyperlipidemic effect of leaf powder and seed oil of the plant was reported. [43, 44] There was not found any significant change in glycosylated haemoglobin. Mild increase in serum insulin levels was observed. There was not found any significant change in lipid profile and liver function test. Significant reduction in serum uric acid and urea were found. Little reduction on serum creatinine level was observed in diabetic rats post 21 day treatment.

Pterocarpus marsupium is known for its pharmaceutical properties like an astringent used in the treatment of dysentery, diarrhea, fever and toothache. [45] It is cited in Ayurveda for curing diabetes and has been reviewed as possessing hepatoprotective and antihyperlipidemic activities. [46] Continuous treatment of crude powder of wood of plant for 21 day proved highly effective in managing hyperglycemia because serum glucose level was found to have normalized. As these effects were only seen in *Pterocarpus marsupium* treated diabetic rats but not in hyperglycemic rats, it was concluded that glucose lowering effect might be due to *P. marsupium* extract. In addition to this, serum insulin levels increased in *Pterocarpus marsupium* treated group. This effect may be because of insulin release through stimulation in β-cell regeneration. Hypoglycemic activity *P. marsupium* extract in experimental diabetic rats was previously reported. [47, 48] Oral treatment with *Pterocarpus marsupium* improved the fasting blood glucose and glycosylated haemoglobin. Previous studies by Sheela and Augusti[49] demonstrate similar results too. Mild lipid lowering effect and significant renal function test were observed in diabetic rats. An increased activity of serum ALT, AST and T-bilrubin were observed in diabetic rats. Oral treatment with *Pterocarpus marsupium* normalized these enzyme activities. Similar results on hepatoprotective effect of stem bark of *Pterocarpus marsupium* on rats were observed by Mankani et al. [46]

Various plant part of *Coccinia indica* which grown abundantly in India have been widely used in the traditional treatment for diabetes. Every part of this plant is valuable in medicine for ring worm, psoriasis, small pox, scabies and other itchy skin eruptions and ulcers, anti-inflammatory, analgesic, hepatoprotective, antioxidant and antimutagenic activities.[50] Continuous treatment of crude powder of *Coccinia indica* fruit for 21 day significantly lowered the blood glucose levels but no significant lowering in fasting blood glucose. Antidiabetic activity of stem of *Coccinia indica* and hypoglycemic activity of leaves of the plant reported. [51, 52] Treatment of plant part prevented the elevation of glycosylated haemoglobin in comparison to control diabetic rats. Treatment of *C. indica* increases the serum insulin level as compare to control group. Increase in triglyceride, total cholesterol and LDL-cholesterol and decrease in HDL cholesterol were observed in diabetic group. Treatment of plant part prevented the elevation of lipid profile significantly in comparison to control diabetic rats. Crude

powder of plant part also showed mild reduction in urea and significant reduction in uric acid and creatinine after treatment. The increased levels of transaminases which are active in the absence of insulin because of the availability of amino acids in the blood of diabetics are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. [53] The restoration of AST and ALT to their normal levels by the crude powder of *Coccinia indica* may also indicate the revival of insulin secretion to near normal levels.

Shilajit, described as India's wonder drug, is used in Ayurveda, the traditional Indian system of medicine. It helps in metabolism, stimulates our energy levels, fights against diabetes and regulates blood sugar balance. Continuous treatment of the extract of Shilajeet for the period of 21 days produced significant tolerance in blood sugar levels of diabetic rats. Treatment with Shilajeet lowered the fasting blood glucose, glycosylated haemoglobin and significant increase in serum insulin levels. Shilajeet improves serum lipid profile as it reduces triglyceride, total cholesterol and significant decrease in LDL-cholesterol while slight increase in HDL-cholesterol. Trivedi et al., [14] also reported effectiveness of Shilajeet in controlling blood glucose level and also improves the lipid profile. Serum urea, uric acid and creatinine levels significantly lowered with the chronic treatment with Shilajeet. This showed that exudates of Shilajeet may help in improving secondary complication like kidney damage in diabetes mellitus. Shilajeet had mild effect on liver enzymes like ALT and AST but significantly lower serum T-bilrubin level.

The polyherbal formulations are proved effective for the treatment of various disorders then the single drug treatment. [54] After 14th and 21st day post treatment animals were subjected to oral glucose tolerance test (OGTT) which directly measures the action of endogenous insulin in response to a glucose stimulus. [55] However, this method does not allow a separate evaluation of β islet cells and peripheral insulin sensitivity of the tissues. Our data suggest that diabetic animals have higher levels of glucose after 120 min of glucose load. Lack of or reduced levels of insulin may be responsible for this observation. However, diabetic animals treated with crude plant powders shows better tolerance then diabetic animals but the standard formulation 'Madhusunya' shows pronounced tolerance then individual to oral glucose load. In combined form, the extracts tend to complement each other thereby producing the desired normoglycemia. This observation may buttress the proposition of Tiwari and Rao[56] as per advantage of polyherbal therapies over monotherapy. Treatment with polyherbal formulation 'Madhusunya' showed significant decrease in fasting serum glucose levels which was near to healthy control. Decrease in blood sugar levels was found to be more effective with polyherbal formulation 'Madhusunya' at the dose of 100 mg/kg as compare to single plant powder at the same dose. The antidiabetic plant extracts may involve one or more compounds which decrease blood glucose levels suggesting that the natural constituents could act synergistically to induce a hypoglycemic effect as described by Marles and Fransworth.

It was observed in diabetic patients the level of glycosylated haemoglobin increased.[57] During diabetes excess glucose present in blood react with haemoglobin so the total haemoglobin level decreased in diabetic rat. Administration of formulation for 21 days prevents a significant elevation in glycosylated haemoglobin in diabetic rats. This could be due to result of improved glycemic control. Earlier studies have shown that in STZ-induced diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles.[59] The mechanisms responsible for the development of hypertriglyceridemia in uncontrolled diabetes in humans (possibly in insulin deficient STZ-diabetic rats) may be due to a number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver.[60] In diabetic rats, there is a decrease in lipoprotein lipase activity resulting in impaired clearance of VLDL and chylomicrons from plasma.[61, 62] In our study, treatment with 'Madhusunya' powder suspended in 1% Gum Acacia solution significantly decreased both serum cholesterol and triglyceride levels in diabetic rats. There were also significantly decreased serum LDL levels by treatment with 'Madhusunya' compared to diabetic control animals. It also produced significant change in serum HDL level by treatment compared to diabetic control group. This might be due to the reduced hepatic triglyceride synthesis or reduced lipolysis that might be due to increase in serum insulin levels in the formulation treated groups. Crude powders of individual plant parts-treated animals have also beneficial effects on lipid profile but the result is more significant in combination. The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol, triglycerides, LDL-cholesterol and raise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions.[63]

Kidney maintains optimal chemical composition of body fluids by acidification of urine and removal of metabolite waste such as urea, uric acid and creatinine.[64] In renal disease, the concentrations of these metabolites increase in blood. Elevated uric acid and creatinine are the significant renal markers and related to renal dysfunction in diabetic hyperglycemia.[65] Our data showed that serum urea, uric acid and creatinine levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triacylglycerol and

cholesterol levels. Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid.[66] Our data showed that Chronic treatment with 'Madhusunya' powder and individual plant parts for 14 days significantly decrease in elevated serum urea, uric acid and creatinine levels in diabetic rats but the effect is more pronounced in formulation, which indicated its beneficial effects on kidney.

Liver enzymes such as AST, ALT and T-bilrubin are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels. [67, 68] The increase in the activities of AST, ALT and total-bilrubin in plasma may be due to the leakage of these enzymes from the liver cells into the blood stream indicated on the hepatotoxic effect of streptozotocin.[64] Continuous treatment of formulation lowered the serum AST, ALT and T-bilrubin levels in diabetic rats. Formulation showed maximum Lowering in hepatic parameter then individual one.

Higher potency of the crude drug is brought about by the synergistic effect among its component compound, even though the potency of each compound is weak when used alone. This is one reason why Madhusunya formulation composed of several crude drug.[69] According to Tiwari and Rao[56], polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects.

Some of the plant powders which showed blood glucose lowering activity on streptozotocin-induced diabetic rats and did not cause any marked lowering in normoglycemic rats like the crude powder *Ocimum sanctum* and *Coccinia indica*. It means that the mixture caused blood glucose lowering of diabetic rats but not in the normal rats therefore it is beneficial for the point of drug development view. The combined mixture of plant powders including Shilajeet showed promising effects either may be due to synergistic effect or herb-herb interaction. Many polyherbal formulations such as Dihar, D-400, Trasina, Diasulin and Diamed have been shown for their antidiabetic and other effects i.e., antioxidant effect, antihyperlipidemic effect etc. Ayurvedic remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combination with immune modulators, diuretics, antioxidants and detoxicants. Immune process plays a predominant role in the destruction of beta cells and features predominantly in the progression of the disease and its secondary complications. The overall studies suggest that the much improved lowering in hyperglycemia following treatment with polyherbal formulation madhusunya over single plant could be due to the combined or synergistic effects of the potential bioactive components that are present in the constituent plants.

Acknowledgements: Two of us (Akansha Mishra and Rohit Srivastava) are grateful to Council of Scientific and Industrial Research, New Delhi (India) for providing Senior Research Fellowships. This paper bears CSIR-CDRI communication number 8553.

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Source of Support: Nil, **Conflict of Interest:** None.