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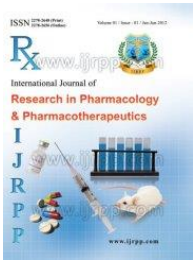

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Evaluation of hypoglycaemic activity of shilajit in alloxan induced diabetic rats

Ravi H Kudthni [1] , Prasad S R[2]* , Basavanna P L[3]

[1]*Assistant Professor, Department of Pharmacology, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, India.*

[2]*Associate Professor, Department of Pharmacology, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, India.*

[3]*Professor, Dept. of pharmacology, Mysore Medical College and Research Institute, Mysore, India*

***Corresponding author: Prasad. S. R**

Email: prasadrudresh@gmail.com

ABSTRACT

Background

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide. This disease constitutes a major health concern affecting about 2.8% of the global population. Currently available synthetic antidiabetic agents produce serious side effects. This leads to a demand for herbal products with antidiabetic activity and fewer side effects. Hence shilajit a herbo-mineral substance with a wide spectrum of biological activity is being evaluated for its anti diabetic activity.

Aim of the study

To evaluate the hypoglycemic activity of shilajit in alloxan induced diabetic rat model and to compare hypoglycaemic activity of shilajit with standard drug, glibenclamide.

Materials and Methods

Alloxan induced (150mg/kg.i.p) diabetic rats were divided into six groups of six animals each weighing 150-200 gms. Group I - Diabetic control: Normal saline. Group II - Standard: Normal saline + Glibenclamide (5mg/kg). Group III – Normal saline + Shilajit dose D1 50mg/kg, Group IV- Normal saline + Shilajit dose D2 100mg/kg, Group V- Normal saline + Shilajit dose D3 150mg/kg, Group VI- Normal saline + Shilajit dose D2 100mg/kg+ Glibenclamide (5mg/kg). All drugs are given once daily orally for 21 days and fasting blood glucose was estimated from blood taken from rat tail vein using glucometer on 0, 7th, 14th, 21st days.

Results

Shilajit by itself has significant hypoglycemic effect. Its hypoglycemic effect is comparable to glibenclamide when used in doses 50mg/kg, 100mg/kg, but when used in dose of 150mg/dl it showed lesser effect. Shilajit when combined with glibenclamide showed potentiated hypoglycemic effect.

Conclusions

The result in this study suggests that shilajit can be used as monotherapy in doses 50mg/kg, 100mg/kg and complementary medicine with glibenclamide for diabetes mellitus.

Keywords: Diabetes mellitus, Albino rats, Alloxan monohydrate, Fasting blood sugar, Glibenclamide, Shilajit.

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia 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]

Diabetes is an "iceberg" disease. Although increases in both the prevalence and incidence of type 2 diabetes have occurred globally, they have been especially dramatic in societies in economic transition, in newly industrialised countries and in developing countries. Currently number of cases of diabetes worldwide is estimated to be around 150 million. This number is predicted to double by 2025.

The results of prevalence studies of diabetes mellitus in India were systematically reviewed with emphasis on those utilizing the standard WHO criteria for diabetes diagnosis. The prevalence of disease in adults was found to be 2.4 per cent in rural, 4.0-11.6 percent in urban dwellers. High frequencies of impaired glucose tolerance, shown by those studies, ranging from 3.6-9.1 percent, indicate the potential for further rise in prevalence of diabetes mellitus in the coming decades. [2]

The number of individuals with diabetes is rising rapidly throughout the world. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities. However, the major sources of the morbidity of diabetes are the chronic complications that arise from prolonged hyperglycemia, including retinopathy, neuropathy, nephropathy, and cardiovascular disease. [3]

Presently used categories of oral anti diabetic agents are- Insulin secretagogues: Sulfonylureas, meglitinides; Non-sulfonylureas: biguanides, thiazolidenidiones & α-glucosidase inhibitors, Dipeptidyl peptidase IV inhibitors, Bile acid sequestrants. The beneficial effects of these are well documented but not without side effects & also patients develop resistance over a period of time. Insulin is used when hyperglycemia is not controlled with oral anti diabetic agents alone. But it can cause hypoglycemia which is the most frequent and potentially the most serious reaction. It also results in local reactions like swelling, erythema, stinging which occur usually at the beginning, but over long usage it can result in lipodystrophy. Therefore there is a need to continue the search for more effective & safer oral anti diabetic agents for the treatment of DM.

Long before the use of insulin became common, indigenous remedies were used for treatment of diabetes and hyperlipidemia. There has been an increasing demand from patients for the use of natural products with antidiabetic activity. [4].

Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of DM, investigations on hypoglycemic agents derived from medicinal plants have gained momentum.[5, 6] The present study aims at investigating the hypoglycemic effect of shilajit in alloxan induced diabetic rat model.

MATERIALS AND METHODS

Materials used in this study are as follows

Chemicals

Alloxan monohydrate, Normal saline, Glibenclamide and Shilajit

Animals

Albino rats of either sex weighing 150 – 200 gm were randomly selected from central animal house facility of Mysore Medical College and Research Institute, Mysore.

Equipments

Mouth gag, Polythene tube, Tuberculin syringe and Glucometer.

Chemicals

Alloxan monohydrate [7, 8, 9, 10, 11]

Alloxan monohydrate 5% solution, dissolved in normal saline was used in this study at the dose of 150mg/kg to induce diabetes in rats.

Structure

2, 4, 5, 6-Tetraoxypyrimidine 5, 6-dioxyuracil.
It is clearly soluble in water.
It is stored between 2-8^{0}c. [12]

Glibenclamide

Glibenclamide belongs to the second generation sulfonylureas. It was taken as a standard drug to compare it with the test drug. [13, 14]

Shilajit

Shilajit was obtained from Kaaya Chikitsa (Department of Medicine), Government Ayurveda College, Mysore.

Animals

Animals used were albino rats of Wistar strain weighing between 150-200gms of either sex. The animals were divided into 6 groups of 6 animals each. The animals were fed with food and water ad libitum.

EQUIPMENTS

Mouth gag

This was used to facilitate the introduction of polythene tube thereby to facilitate oral feeding in rats.

Polythene tube

Small polythene tube was used for the oral administration of the standard drug and the test drug.

Tuberculin syringe

A tuberculin syringe of 1 ml size was used for injecting alloxan monohydrate into the peritoneal cavity of rats to induce diabetes in them.

Glucometer

The glucometer used in this study was mini smart care from TaiDoc Technology corporation.

Method of using glucometer

To the smart care glucometer, strip of same company is inserted into the slot provided. Glucometer displays the code number. It has to match with the code of test strip. This can be changed with the help of a switch provided for changing of the code. Once the code matches, the glucometer will indicate to apply the blood drop to the strip, to the place provided for it. After 5 seconds of application of blood, it shows the blood sugar level. The fresh arterial, venous and capillary blood is used.

The chemical method was employed here to study the hypoglycemic activity. Alloxan monohydrate was used to induce diabetes. Animals used were albino rats with body weight between 150-200gms of either sex. [11] The animals were procured from animal house of Mysore Medical College and Research Institute, Mysore, after taking Institutional Animal Ethics Committee approval. Animals were fed with pellet diet and water throughout the experiment. Animals were acclimatized to laboratory conditions before carrying out any experimental work. Glucometer was used to record the blood sugar. For measuring FBS, blood was collected from the rat's tail vein. [7]

Induction of diabetes

Animals were injected intraperitoneally with freshly prepared alloxan monohydrate 5% solution dissolved in normal saline in a dose of 150 mg/kg body. [15] Following injection, animals were carefully observed for the first 24 hrs for any evidence of allergic reaction, behavioral changes and convulsions. No untoward reaction was observed in any animals. Fasting blood glucose was recorded daily morning for one week. Animals developed stable hyperglycemia after 4-5 days. Only those animals with blood glucose more than 200mg/dl were selected for the study.[15,16] Later they were divided into 6 groups, each group having 6 animals. The 6 groups were named as diabetic control, standard, test drug D1, test drug D2, test drug D3 and Standard + test drug D2 respectively.

Administration of drugs

Each group of animals was orally fed with the following agents.

GROUP I - Diabetic control

Consists of 6 rats treated with alloxan (i.p.), normal saline (oral) for 21 days.

GROUP II- Standard

Consists of 6 rats treated with alloxan (i.p.), normal Saline and glibenclamide 5 mg/kg (oral) for 21 days.

GROUP III - Test drug D 1

Consists of 6 rats treated with alloxan (i.p.), normal saline and shilajit Dose (D1) (oral) for 21 days.

GROUP IV - Test drug D 2

Consists of 6 rats treated with alloxan (i.p.), normal Saline and shilajit Dose(D2) (oral) for 21 days.

GROUP V- Test drug D 3

Consists of 6 rats treated with alloxan (i.p.), normal saline and shilajit Dose (D3) (oral) for 21 days.

GROUP VI

Standard and consists of 6 rats treated with alloxan (i.p.), normal saline, shilajit Dose (D2) and glibenclamide 5mg/kg (oral) for 21 days.

Diabetic Control

Diabetic animals of this group were kept for overnight fasting and their FBS was recorded before giving any drug. This value was considered as FBS value for day zero. Now the animals of this group were orally fed with 0.5ml normal saline. Thus normal saline was orally given in the morning, every day for 21 days. FBS was recorded on the 7^{th}, 14^{th} and 21^{st} day. [4] Normal saline is given in the control group, because it is an inert substance and it is the vehicle for administration of alloxan and shilajit. Hence it does not produce any effect on blood glucose level. The FBS values of these animals serve as control to compare with FBS values of standard and test group.

Standard

After recording the FBS values for day zero, these animals were orally fed with 0.5ml of normal saline + Glibenclamide 5mg/kg[17], in the morning every day for 21 days. FBS values of this group were recorded on the 0^{th}, 7^{th}, $14^{th}$and 21^{st} day, to see the reduction in blood glucose level produced by glibenclamide over a period of 21 days (as it is a standard oral hypoglycemic drug).

Test drug D 1

After recording the FBS values for day zero, these animals were orally fed with 0.5ml of normal saline + shilajit 50mg/kg in the morning of every day for 21 days. FBS values of this group were recorded on the 0^{th}, 7^{th}, 14^{th} and 21^{st} day; to see the whether there is a fall in blood glucose level.

Test drug D 2

After recording the FBS values for day zero, these animals were orally fed with 0.5ml of normal saline + shilajit 100mg/kg, in the morning of every day for 21days. FBS values of this group were recorded on the 0^{th}, 7^{th}, 14^{th} and 21^{st}, to see whether there is further fall in blood glucose level with this combination.

Test drug D 3

After recording the FBS values for day zero, these animals were orally fed with 0.5ml of normal saline + shilajit 150mg/kg, in the morning of every day for 21days. FBS values of this group were recorded on the 0^{th}, 7^{th}, 14^{th} and 21^{st}, to see whether there is further fall in blood glucose level with this combination.

Standard+ Test drug D 2

After recording the FBS values for day zero, these animals were orally fed with 0.5ml of normal saline + Glibenclamide 5mg/kg + shilajit 100mg/kg, in the morning of every day for 21 days. FBS values of this group were recorded on the 0^{th}, 7^{th}, 14^{th} and 21^{st}, to see whether there is further fall in blood glucose level with this combination.

Thus hypoglycemic activity of shilajit was assessed for 3 doses alone and shilajit 100mg/kg in combination with glibenclamide. Its capacity to reduce the blood sugar level is compared with standard drug glibenclamide.

Statistical Analysis

The results have been analyzed by calculating the mean values, the standard deviation, and the analysis of variance (ANOVA) followed by Tukey's multiple comparison test to show the difference between groups. P value < 0.05 is considered as significant.

RESULTS

The results of our study showed that in alloxan-treated rats, the rise in blood glucose level remained stable throughout the study period rendering them diabetic. There was a significant reduction in blood glucose level with Shilajit, when compared to the control. Reduction in blood glucose level shown by the Shilajit in doses 50mg/kg and 100mg/kg was

statistically significant ($p < 0.001$) when compared with the control.

The combination of shilajit100mg/kg with Glibenclamide showed extremely significant reduction ($p< 0.001$) in blood glucose level throughout the experiment, when compared with the control. Shilajit 150mg/kg produced no significant reduction in blood glucose level compared with Shilajit 50mg/kg, 100mg/kg alone. **(**See **Table 1)**

Table 1: Effect of shilajit on blood glucose level in diabetic rats.

GROUP	TREATMENT	Day 0	Day 7	Day 14	Day 21
I Diabetic control	Alloxan +normal saline	261.6±23.7	260.1±24.7	260±24.3	258.1±24.1
II Standard	Glibenclamide5 mg/kg	278.8± 35.6	165.6± 13.3*	134 ±15.2***	117.33 ±14***
III Test D 1	shilajit(50mg/kg)	235.6 ± 5.4	125.1± 6.4**	120.3±4.2**	93.8±4.5***
IV Test D 2	Shilajit (100mg/kg)	406.6±60.6	280.6±48.6*	245.6±37.6**	244. 5±35.6***
V Test D 3	Shilajit (150mg/kg)	293.1±54.5	247.8±46.9	232.5±42.6	245.3±58.5
VI Standard + Test D 2	Shilajit (100mg/kg) + glibenclamide 5mg/kg	256.5±8.7	131.6±15.7**	133±14.1***	127.6±13.8***

Note: Values are expressed as Mean±S.E.M (n=6)
* $p < 0.05$ is considered as significant.
** $p < 0.01$ is highly significant
*** $p < 0.001$ is extremely significant

DISCUSSION

Diabetes mellitus is one of the commonest chronic illnesses to human beings. It is characterized by deleterious hyperglycemia and is one of the leading diseases in the world. [18] The world health organization estimates that by the year 2030, the number of people with diabetes will have reached 370 million [19]. There is a high level of treatment failures and unpleasant side effects associated with oral anti diabetic drugs generating an urgent need and desire for alternative treatment by the use of plant based products are becoming popular in the treatment and management of diabetes. [20]

Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicine alone fails. Many herbal extracts are known to have hypoglycemic effects.

The current study was performed to evaluate the hypoglycemic effect of shilajit a herbomineral substance, in alloxan induced diabetic rats. This study shows that shilajit produces a marked decrease in blood glucose levels in alloxan induced diabetic rats.

The present study goes in accordance with the previous study by Trivedi NA et al with respect to the outcome. [17] It shows that shilajit has hypoglycemic activity and its activity is comparable to the standard drug glibenclamide which is statistically very significant. Bhattacharya S.K et al study, shilajit attenuates streptozotocin induced diabetes mellitus and decrease in pancreatic islet superoxide dismutase activity in rats, supports our study. [21]

Our study shows that glibenclamide and shilajit reduced the blood glucose levels and the effect of shilajit is comparable to glibenclamide in alloxan induced diabetic rats.

The combination of shilajit100mg/kg with glibenclamide showed extremely significant reduction in blood glucose level throughout the experiment, when compared with the control and shilajit 150mg/kg but no significant reduction was found compared with shilajit 50mg/kg, 100mg/kg alone.

The analysis gives us the result that Shilajit by itself has significant hypoglycemic effect on long term use in doses 50mg/kg, 100mg/kg. Although shilajit 150mg/kg also produced reduction in FBS but it is statistically not significant. But hypoglycemic

effect produced by shilajit 50mg/kg and 100mg/kg is comparable to the standard drug glibenclamide. Shilajit 100mg/kg when combined with glibenclamide showed significant hypoglycemic effect. The combination effect is not statistically significant when compared to the standard alone although the combination reduced FBS more than single drugs used. This suggests the combination has slightly potentiated effect when compared with the standard drug alone.

The exact mechanism of action is still unclear. Experimental diabetes is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocytes in the inflammatory focus. These cells might be the source of the cytotoxic oxygen radicals. Shilajit has been reported to reduce macrophage and lymphocyte activation and migration, as a part of its immunomodulatory activity. [21] Moreover, being an antioxidant it will prevent damage to the pancreatic islet cell induced by the cytotoxic oxygen radicals. [21, 22, 23] However the exact mode of action still needs to be elucidated and requires further studies in both animal models and in human trials.

LIMITATIONS OF THE STUDY

The study is very primitive in the parameters used. The study has been carried out only in one species of animals viz "rats" and needs to be extended to other animals as well. Only the fasting blood glucose was estimated in this study which does not give a clear picture about the effect of shilajit on other parameters of diabetes mellitus.

The effect on serum lipid levels, reactive oxygen species, atherogenesis β-cell pathology etc needs to be evaluated. Owing to non availability of analytical methods in our set up, the pharmacokinetic profile has not been identified.

Acute toxicity testing has been fairly preliminary. Chronic toxicity studied to evaluate the effect of extract of shilajit on various haematological parameters, lipid profile, electrolyte profile, teratogenic and carcinogenic potential etc. have to be undertaken for further evaluation.

Pharmacokinetic and pharmacodynamic differences always exist between human and animal species. Hence to confirm the above result it needs further evaluation in human subjects.

CONCLUSIONS

The study on evaluation of hypoglycemic activity of Shilajit in alloxan induced diabetic rat has shown the following effects.

1. Shilajit by itself shows the hypoglycemic effect comparable to the standard drug glibenclamide in doses 50mg/kg and100mg/kg but has lesser effect in dose of 150mg/kg.
2. Shilajit given along with glibenclamide the hypoglycemic activity was potentiated. Shilajit may have potentiated the action of glibenclamide or vice versa.

These findings suggest that shilajit can be used as a monotherapy or complementary medicine with glibenclamide in diabetes mellitus.

However animal studies cannot be directly compared with effects on humans. So there is a need for clinical evaluation of shilajit in humans to confirm the effect.

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