ISSN 2394 - 7837

ARTICLE

Hypoglycemic Activity of Natural Product Shilajit on Streptozotocin Induced Diabetic Mice, *Mus musculus*.

Jasmi. A and Jeeja kumari V.K*

Received 04/03/2018 Accepted 15/04/2018

Abstract

Diabetes Mellitus is a group of heterogeneous metabolic disorders characterized by high blood glucose concentration result from a defective insulin secretion or activity. Based on the current trends, around 360 million individuals will have diabetes by the year 2030. The use of ethnobotanicals has a long folkloric history for the treatment of blood glucose lowering abnormalities. In the present study, Natural product Shilajit extract was selected for antidiabetic evaluation owing to its ethnomedicinal use in curing diabetes. Shilajit aqueous extracts were prepared and administered by oral guage at a dose of 100, 500mg/kg body weight for a period of 15 days to streptozotocin (150mg/kg body weight) induced diabetic mice. Water intake, food intake and body weight of diabetic mice were recorded. After completing the experimental period, the blood samples were collected from the mice and the serum glucose was estimated using One Touch Glucometer. Biochemical parameters viz, total protein and lipid profile were determined using appropriate diagnostic kits. The result shows that the oral administration of Shilajit extract (500 mg/kg, 100 mg/kg body weight) for 15 days showed significant improvement in body weight in diabetic mice compared with untreated diabetic mice. Glucose level of Streptozotocin induced diabetic mice was significantly decreased ($P< 0.000$) by day 15th after administration of 100 and 500mg/kg body weight.Thus the present investigation confirms the antidiabetic activity of aqueous Shilajit extract on streptozotocin induced diabetic mice.

Keywords: *Mus musculus,* antidiabetic, Shilajit, Streptozotocin, serum glucose

Introduction

Diabetes mellitus is heterogeneous metabolic disorder characterized by hyperglycemia due to impaired insulin secretion and aberrant glucagon secretion resulting from changes in pancreatic islet cell function. Diabetes is a disease that acquires epidemic form, as its prevalence has five folded during the last fifteen years and constitutes one of the major threats to human health in 21stcentury (Zimmet P et al.,2001). The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes (W.Marshal and S.K Bangret,2004). Several studies have proved that Diabetes is a key factor contributing to the increase in solid organ malignancies or tumor including liver, pancreas, colorectal, breast, endometrial, uterine, and bladder (Huxley R, et al.,2005). Hyperglycemia is considered a primary cause of diabetic vascular complications and is associated with oxidative stress, impaired trace element and lipid metabolism as well as pancreatic enzyme abnormalities (Opara EC et al., 1999).The commonly practiced treatment of diabetes includes oral antidiabetic drugs, insulin injection and management through diet and physical exercise. Oral hypoglycemic agents, currently used have serious side effect hence there is a need to search a newer antidiabetic agents that having high therapeutic efficacy with minimum side effect (Holman RR and Turner RC,1991). Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of diabetes. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations

Shilajit is considered one of the traditional herbo-mineral drugs in Ayurveda. It is a thick, blackish-brown mineral pitch resin that oozes out of cracks in the Himalayan Mountains as the summer heat raises the temperature of the rock (Kong YC et al.,1987& Srivastava RS et al.,1988). The major physiological action of shilajit was found to be due to the presence of the bioactive dibenzoalpha-pyrones along with humic acid and fulvic acids which acted as carrier molecules for the active ingredients (Ghosal S. (1990). Other molecules present in Shilajit preparations are eldagic acid, some fatty acids, resins, latex, gums, albumins, triterpenes, sterols, aromatic carboxylic acids, 3,4-benzocoumarins, amino acids, polyphenols, and phenolic lipids (Kong YC et al.,1987). It is considered to be an important herbal supplement that encourages normal body functioning and

* P G and Research Department of Zoology, Sree Narayana College, Kollam, Kerala.
*Corresponding Author email: jasmi.aboobaker123@gmail.com

acts as a general body toner (Acharya SB et al.,1988). Shilajit protects and enhances the workings of the kidneys, pancreas and thyroid gland. Shilajit has an important and unique place in traditional texts such as Ayurveda, Siddha and Unani medicine. Shilajit is prescribed to treat genito-urinary disorder, jaundice, gallstone, digestive disorders, enlarged spleen, epilepsy, nervous disorder, chronic bronchitis, anemia (Bhattacharya SK and Sen AP (1995). Fulvic Acid, the major ingredient of the Shilajit, plays a vital role in penetrating the cell walls and transporting the minerals deep into the cells. Thus it supplies essential minerals thereby ensuring that the vitamins too are assimilated. This restores optimum functioning of cells and energy metabolism (Trivedi et al.,2004).Shilajit is said to be able to reduce pain, treat all types of arthritis, treat joint and muscle pain and treat inflammation. It can also promote better concentration and is thought to increase learning ability and enhance memory. Therefore, the present investigation was undertaken to study the antidiabetic effect of aqueous Shilajit extract on streptozotocin induced diabetic mice.

Materials and Methods

Herbal material and preparation of extract

Shilajit is the most potent rejuvenator and anti aging block buster ever known to the mankind. It cannot be used directly in the raw form and further extraction is needed. Shilajit was extracted with double distilled water for 30 minutes in boiling water bath. It was filtered using Whatman's No. 40 filter paper and the filtrate was stored in a cool place. The extract was administered at a dose of 100mg & 500mg per kilogram body weight.

Experimental animals

3-4 months old adult virgin male mice weighing 30-35gm were used for the experiment. Animals are collected from animal house of Department of Zoology, university of Kerala. They were maintained under standardized animal housing conditions (temperature 25 ± 2°C facilities with 12h light/ dark cycle) with free access to standard pellet diet and water throughout study. They were housed in standard cages made of polypropylene and provided with bedding or nesting materials. Animals described as fasted were deprived of food for 16 h, but had free access to water.

Induction of experimental diabetes

Diabetes was induced by intraperitoneal administration of streptozotocin (150mg/kg body weight) dissolved in normal saline to the male Swiss albino mice, after an overnight fast (access to only water) of 16 hours. Fourteen days after streptozotocin injection, mice with blood glucose level greater than 140mg/dl were separated and used for the study.

Experimental design

Experiments were designed in line with the objectives of the study. In order to trace the curative effect, Shilajit was fed after the mice were made diabetic by Streptozotocin administration. Standard pellet diet and water was provided to the animals.

Experimental groups

Animals were divided into four groups of five mice each.

Group I - served as Normal healthy control
Group II - served as Streptozotocin diabetic control
Group III - treated with Shilajit 100mg/kg body weight.
Group IV - treated with Shilajit 500mg/kg body weight.

Sampling of Animals

After experimental period, the animals were anaesthetized by intravenous injection using thiopental sodium (100mg/kg body weight) and blood samples were carefully collected by cardiac puncture in eppendorf tubes and the serum was separated from blood samples carefully using ultracentrifuge. The liver, kidney and pancreatic tissues were removed and weighed and kept for histological studies. The tissues were rinsed in cold saline (0.9%)and preserve in10% buffered formalin and Bouin's fluid for further histological processes.

Symptomatic Analysis

The external diabetic symptoms such as water intake, food intake and body weight were studied for a period of 30 days.

Water intake:

Feeding bottle containing 100ml water was inserted into the grid of the cage containing the animals. After 24hr, the bottle was removed from the cage and remaining quantity of water was measured.

Food intake:

A known amount of food was introduced into the cage after removing the bedding material, unfed was collected after 24hr and weighed. The amount of food consumed was calculated.

Body weight:

Body weight of all treated groups and control group of mice were recorded before and during treatment period. A properly calibrated and standardized electronic balance was used for taking body weight of the mice and expressed as gram (g).

Biochemical Analysis

The biochemicalparameters measured were fasting blood glucose, total protein, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and triglycerides as per reported methods (Sharma Set al.,2007) using ERBA diagnostic kits using Auto analyzer. All the biochemical parameters were analysed in the different experimental groups on the experimental period.Blood samples were collected from the tail vein after overnight fasted mice. The

blood glucose level in the samples was estimated using One Touch Glucometer (Lifescan, Johnson & Johnson, California) and results were demonstrated in mg/dl.

Histological Preparation

Tissues for histological studies were obtained from euthanized Swiss albino mice. Viscera were cut opened and liver, kidney and pancreatic tissues were taken out and preserved in buffered formalin and Bouin'sfluid. Three day fixation was needed for the tissues fixed in Bouin's fluid and one day fixation for tissue in buffered formalin.

Statistical Analysis

All the data were subjected to one way analysis of variance [ANOVA] followed by Duncan's multiple range test to determine the level of significance between the control and treatment means in different groups. Statistical analysis was performed using the SPSS statistical software package.

Results

The effect of Shilajit extract on body weight of Streptozotocin induced diabetic mice are given in Table:1. A significant difference in body weight gain was observed between Shilajit extract groups and the Streptozotocin control group during the experimental period. Oral administration of Shilajit extract (100mg/kg body weight) for 15 days showed significant improvement in body weight (Table: 1). Oral administration of Shilajit extract (100mg/kg) for 15 days showed significant improvement in body weight.

Shilajit 500mg/kg body weight shows maximum water intake compared to normal control.

Fasting Plasma Glucose level in streptozotocin control group was significantly increased compared with the normal control group ($p<0.00$). Administration of Shilajit 500mg/kg body weight shows significant decrease in the plasma glucose level ($p<0.000$).

The serum protein level of streptozotocin control mice showed a significant decrease ($p<0.05$) when compared with normal control mice. Shilajit extract treated groups showed significant increase ($p<0.05$) in serum protein level compared to streptozotocin control. 500mg/kg body weight Shilajit offered a significant protection against alteration in serum protein level. (Table: 4).

A significant augmentation in triglyceride, and total cholesterol ($P<0.000$) levels were observed in diabetic mice as compared to normal control. After treatment with Shilajit extract there was significant increase in ($P<0.000$) plasma HDL level and decrease in total cholesterol, LDL, VLDL and triglycerides (Table: 5). The continuous treatment with Shilajit 500mg/kg extract brought down the lipid profiles in diabetic mice to almost normal levels.

Histology Analysis

The pancreatic cells of control groups displayed normal histological features: the islets are oval and compactly arranged, no inflammatory cells were observed. In the streptozotocin control mice, small volumed and irregularly shaped islets with a reduced number of β-cells, and disarranged and vacuolization of the cytoplasm. The size and cell number of islets were increased after administration of 100, 500mg/kg Shilajit extract, which led to formation of clear islet edge and orderly arranged cells.

Table 1. Effect of Shilajit on body weight

Sl No.	Groups	Body weight (gm)		
		1st day	7th day	15th day
1	Normal control	33.4	34.16	34.93
2	Streptozotocin control	33.13	31.46	30.53
3	Streptozotocin+ Shilajit 100	30.78	32.32	32.56**
4	Streptozotocin+ Shilajit 500	28.86	29.6	30.12

Table 2. Effect of Shilajit on food and water intake

Sl No.	Gropus	Food(gm)	Water(ml)
1	Normal control	6.41	6
2	Streptozotocin control	3.33	2
3	Streptozotocin + shilajit100	3.64	3.3
4	Streptozotocin + shilajit500	3.92	8

Table 3. Effect of Shilajit on Blood glucose level

Sl No.	Groups	Blood glucose(mg/dl)	
		Initial	Final
1	Normal control	72.87±0.075	75.10±0.48
2	Streptozotocin control	216.35±0.776	322.01±0.52***
3	Streptozotocin + shilajit 100	220.20±0.92	194.63±0.53
4	Streptozotocin+ shilajit 500	292.85±0.85	247.75±1.73***-

Table 4. Effect of Shilajit on total protein

Sl No.	Groups	Serum total protein(mg/dl)
1	Normal control	9.34±0.21
2	Streptozotocin control	7.1±0.06
3	Streptozotocin+ shilajit 100	8.56±0.11
4	Streptozotocin+ shilajit 500	8.77±0.19

Normal control mice liver contain hexagonal lobules with number of regular hepatocytes with distinct central vein, polygonal hepatocytes arranged in strands running radially from the central vein with blood sinusoids in between these hepatic strands. Whereas in diabetic mice lumen of central vein extensively filled with fibrous tissue. They shows marked necrosis, degeneration of hepatocytes and congestion of central vein. However in diabetic mice treated with 100mg/kg, 500mg/kg Shilajit extracts the liver looked almost normal with central vein and lesser vacuolization, necrosis of hepatocytes and disappearance of congestion in blood sinusoids.

The normal control mice kidney contains abundant glomeruli, tubular epithelial cells and Bowman's capsule.

Table 5. Effect of Shilajit on Lipid profile

Sl No.	Groups	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	Triglycerides (mg/dl)
1	Normal control	162.86±0.79	90.10±1.35	50.88±0.68	22.02±0.11	112.12±1.75
2	Streptozotocin control	247.83±0.83	28.44±0.63	161.92±0.65	57.28±0.17	286.30±0.84
3	Streptozotocin+ Shilajit 100	188.25±1.37	37.86±1.83	98.66±1.37	51.73±0.23	243.21±0.76
4	Streptozotocin+ Shilajit 500	121.50±0.82	40.97±1.49	33.61±0.73	46.46±0.08	232.91±0.66

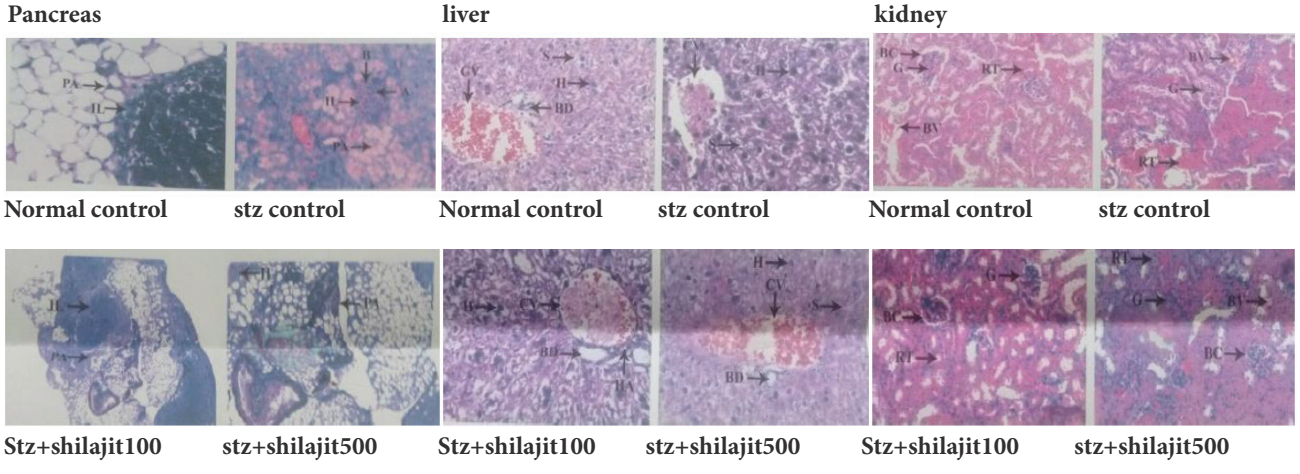

Figure 1. Histopathological changes of pancreas, liver and kidney of both control and experimental mice after treatment period.

PA-Pancreatic acini; A- Alpha cells; B-Beta cells; IL-Islets of Langerhans; CV- Central vein; S-Sinusoids; H- hepatocytes; BD- Bile Duct; BC- Bowman's capsule; G- Glomerulus; RT- Renal tubules; BV- Blood Vessels

The diabetic control mice kidney showed many pathological alterations such as partial cellular lesion with acidophilic material in the glomerulus, concentric fibrosis with distortion of the tubular wall and enlargement of the tubular lumen, vacuolization of the cytoplasm of the tubular epithelium cells; atypical nuclei, and inflammatory processes in the interstitial spaces in renal papilla and pelvis regions. Whereas the diabetic mice treated with 100 mg/kg, 500 mg/kg Shilajit extracts showed atropic glomeruli and regeneration of tubular epithelial cells and reduce tubular lumen enlargement and distortion of tubular wall.

Discussion

In the present study, Natural product Shilajit extract was selected for antidiabetic evaluation owing to its ethnomedicinal use in curing diabetes. The results of the present study indicated that Shilajit extracts reduced the glucose level in streptozotocin induced diabetic mice. Streptozotocin is toxic to the insulin producing beta cells of the pancreas in mammals. It interferes with cellular metabolic oxidative Mechanism. The increase in glucose levels has been attributed to the destruction of β cells by Streptozotocin. Damage to the Beta cells is associated with the liberation of stored insulin after which the insulin synthesis is stopped leading to a persistent diabetic state. Since insulin is no longer available, glucose absorption is impaired leading to hyperglycemia. Long-term treatment with shilajit increases the number of β-cells of pancreas, i.e. pancreatotrophic action, which may result in better sensitivity of pancreatic β cells with prompt secretion of a large quantity of insulin in response to hyperglycemia.

Oral administration of Shilajit extract (500mg/kg, 100mg/kg body weight) showed significant improvement in body weight in diabetic mice.The major physiological action of shilajit was found to be due to the presence of the bioactive dibenzoalpha-pyrones along with humic acids and fulvic acids which acted as carrier molecules for the active ingredients (Ghosal S. (1990). They helps in metabolism and enhances the absorptive and detoxifying capacity of the body.

The total protein level significantly decreased in Streptozotocin induced mice compared to control mice. They cause degradation of normal protein content and hepato toxic condition causes defective protein biosynthesis in liver. The treatment with Shilajit of both concentration (100 mg/kg, 500mg/kg) gradually restored the protein content. Liver is the only organ that can catabolized and excrete quantitatively important amount of cholesterol. Our results showed a significant increase in the total cholesterol levels among the diabetic groups compared to control. Shilajit also produced a significant beneficial effect on the lipid profile by reducing Total Cholesterol, Triglycerides, LDL and VLDL cholesterol level and significantly increasing HDL cholesterol in streptozotocin induced diabetic mice. Shilajit-induced favorable changes in the lipid profile in diabetic mice may due to its direct action on lipid metabolic pathways (Trivedi et al.,2004).

Streptozotocin treated liver, kidney and pancreas shows cellular damage and ultrastructural changes. They shows degeneration of cells and congestion of blood vessels in liver and kidney. Reduce the area of Islets of langerhans and number of cells in pancreas. Shilajit treated liver, kidney and pancreas reduces structural abnormalities and enhances normal functions.

Conclusion

Streptozotocin is well known for its selective pancreatic islets β cell cytotoxicity and interferes with cellular metabolic oxidative mechanism. Our results reported that STZ is capable of causing marked alteration in some biochemical and histological changes in liver, kidney and pancreas. The present study was conducted to assess the hypoglycemic activity of Shilajit extract in STZ induced diabetic Swiss albino mice. Shilajit has been known and used for centuries by the Ayurvedic medicine, as a rejuvenator and as antiaging compound. Streptozotocin induced groups shows decrease in body weight, blood glucose, and HDL Cholesterol levels. The biochemical and histological study of Shilajit treated group (100mg/kg, 500mg/kg) reveals the protective effect of Shilajit against diabetes. The aqueous extracts of Shilajit has antidiabetic activity as it lower serum glucose level in diabetic mice and significantly increase the HDL Cholesetrol level. It also increases body weight of diabetic mice. It may be concluded that Shilajit extract possess hypoglycemic activities and they may be used as hypoglycemic agent. Further studies are necessary to work out the exact mechanism of action involved in the antidiabetic activity of this natural product.

Acknowledgement

The authors would like to express thanks, to Department of Zoology, University of kerala for providing SAP facilities and laboratory facilities.

References

1. Acharya SB, Frotan MH, Goel RK, Tripathi SK, Das PK. (1988). Pharmacological actions of Shilajit. Indian J Exp Biol. 26(10): 775-7.
2. Bhattacharya SK, Sen AP.(1995). Effect of shilajit on biogenic free radicals. Phytother Res;9:56-9.
3. Ghosal S. (1990).Shilajit Part 7- Chemistry of Shilajit, an immunomodulatoryayurvedicrasayana. Pure ApplChem (IUPAC);62:1285-8.
4. Holman RR, Turner RC. (1991). Oral agents and insulin in the treatment of NIDDM. In: Pickup J, Williams G, editors. Text Book of Diabetes. Oxford: Blackwell; pp. 467–469.
5. Huxley R,Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. (2005).Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies.Jun 6;92(11):2076-83.

6. Kong YC, Butt PPH, Ng KH, Cheng KF, Camble RC, Malla SB.(1987). Chemical studies on a Napalese panacea; Shilajit. Int J Crude Drug Res 25: 179–187.
7. Opara EC, Abdel-Rahman E, Soliman S.(1999). Depletion of total antioxidant capacity in type 2 diabetes Metabolism.;48:1414–7.
8. Sharma S, chaturvedi M, Edwin M, Shukla S, Sagrawat H.(2007). Evaluation of the phytochemicals and antidiabetic activity of *Ficus bengalensis.* Int J Diabet; 27(2): 56-59.
9. Srivastava RS, Kumar Y, Singh SK, Ghosal S.(1988). Shilajit, its source and active principles. Proc 16 IUPAC (Chemistry of Natural Products). Kyoto Japan.:524.
10. Trivedi, N.A. Majumder B. Bhatt J.B. Hermavathi K.G.(2004) Effects of Shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian J. Pharmacol.; 36: 373-376.
11. W.Marshal,S.KBangret(2004). Clinical chemistry Elsevier Limited, pp.191-217.
12. Zimmet P, Alberti KG, Shaw J.(2001). Global and societal implications of the diabetes epidemic. Nature.;414:782–787.