IJHAS

ISSN 2278-4292

International Journal of Health & Allied Sciences

www.ijhas.in

Volume 5 / Issue 1 / January-March 2016

Official Publication of JSS University, Mysore

Medknow

Wolters Kluwer

Anti-diabetic activity of *Shilajatvadi Lauha*, an Ayurvedic traditional herbo-mineral formulation

Thakur Rakesh Singh, Laxmi Narayan Gupta[1], Neeraj Kumar[1], Vikas Kumar[2]

Department of Rasa Shastra and Bhaishajya Kalpana Including Drug Research, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, [1]Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, [2]Department of Pharmaceutics, Neuropharmacology Research Laboratory, Indian Institute of Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India

ABSTRACT

Context: *Shilajatvadi Lauha* (SL) is used in Ayurveda as Indian traditional medicine for treating diabetes mellitus. **Aims:** To explore the anti-diabetic potential of SL in nicotinamide-streptozotocin induced diabetic rats. **Materials and Methods:** SL (10, 30, and 100 mg/kg) and glibenclamide (10 mg/kg) were orally administered once daily to diabetic rats for 14 days. Blood glucose, plasma insulin, total cholesterol (TC), triglycerides (TGs), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and very LDL-C (VLDL-C) were examined. **Results:** SL significantly lowered the blood glucose without any hypoglycemic effect on their control counterparts, which was comparable to that of the standard anti-diabetic drug, glibenclamide. SL also showed reduction in the levels of TC, TGs, LDL-C, VLDL-C, but it increases the levels of plasma insulin and HDL-C in diabetic rats. **Conclusions:** SL possesses anti-diabetic and anti-hyperlipidemic activities in Type 2DM rats, which seems to scientifically validate its traditional uses and might be a promising drug in the therapy of diabetes mellitus and its hyperlipidemic complications.

Address for correspondence: Dr. Thakur Rakesh Singh, Department of Rasa Shastra and Bhaishajya Kalpana Including Drug Research, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar - 361008, Gujarat, India.
E-mail: rakeshayu1984@gmail.com

Access this article online	
Quick Response Code:	Website: www.ijhas.in
	DOI: 10.4103/2278-344X.173889

Key words: Anti-diabetic activity, anti-hyperlipidemic activity, diabetes mellitus, herbo-mineral formulation, *Shilajatvadi Lauha*

INTRODUCTION

Diabetes mellitus is associated with impaired glucose metabolism that leads to an increase in free radical production and increase in triglycerides (TGs) and lipoprotein levels with an increased risk of vascular and renal diseases.[1] Therefore, among the various therapeutic strategies, combination of anti-hyperglycemic, anti-hyperlipidemic, and antioxidant activity can be beneficial in the prevention of diabetes mellitus and its complications. Unfortunately, besides having a number of side effects, none of the oral hypoglycemic agents have been successful in maintaining euglycemia and controlling long-term macro and microvascular complications.[2] As a result, there is a growing need for new oral anti-diabetic drugs from traditional medicines as an effective and safe alternative therapy in the management of Type 2DM. Recently, herbo-mineral formulations are preferred as effective oral hypoglycemic agents as they are safe for long-term use, wide range of therapeutics, easily accessible, and cost effective.[3]

How to cite this article: Singh TR, Gupta LN, Kumar N, Kumar V. Anti-diabetic activity of Shilajatvadi Lauha, an Ayurvedic traditional herbo-mineral formulation. Int J Health Allied Sci 2016;5:9-14.

Herbo-mineral formulations are prepared by addition of *bhasmas* (ashes)/*rasayogas* (mineral preparations)/purified minerals and herbal drugs, and finally they get triturated in *khalva yantra* i.e. mortar and pestle. They are popular for quick action, low dosage, good palatability, and long-lasting stability.[4] All these qualities of herbo-mineral preparations show significant importance from other dosage forms. *Shilajatvadi Lauha* (SL) is one of the herbo-mineral formulations which is used in Indian System of Medicine since ancient times for treating diabetes mellitus and other disorders.[5] It contains pure *Shilajit*, *Swarna makshika bhasma* (ash of chalcopyrite), *Shunthi* (*Zingiber officinale* Roscoe), and *Marich* (*Piper nigrum* L). Moreover, *Pippali* (*Piper longum* L) in equal quantity and *Lauha bhasma* (ash of iron-turning) are 6 times to other ingredients.[6] Ancient scholars suggest use of SL in *Kshaya/Rajayakshama* (tuberculosis) as primary indication. *Kshaya* and *Madhumeha* (diabetes mellitus) are debilitating diseases which are mainly caused by degeneration of the body components (*dhatu kshaya*); hence, patients need *rasayana* (Rejuvenation) treatment which balances the body components. SL contains *Shilajit* and *Swarna makshika bhasma* which has already proved anti-diabetic effect,[7,8] *Lauha bhasma* is famous for its *rasayana* activity,[4] and *Trikatu* (*shunthi*, *marich*, and *pippali*) regulate indigestion (*Agnimandya*) which is pioneer cause for imbalances in body components.[9] In our clinical practice, SL was used to treat diabetes mellitus, but there was no scientific data to prove its efficacy. By considering all this points, the present study was 1st time undertaken to evaluate the anti-diabetic potential of SL and its effect on plasma insulin and lipid profile level in nicotinamide-streptozotocin (STZ) induced diabetic rats.

MATERIALS AND METHODS

Animals

Adult Charles Foster male albino rats (150 ± 10 g) were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45–55% relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum*. Experiments were conducted between 09.00 and 14.00 h. Principles of laboratory animal care (NIH publication number 85–23, revised 1985) guidelines were followed. Protocol of the study was approved by Central Animal Ethics Committee (letter number: Dean/13-14/CAEC/327 dated November 20, 2013). Rats were equally divided into groups of six animals each except for control in which 12 rats were taken.

Preparation of *Shilajatvadi Lauha*

SL comes under formulation of *Churna Lauha kalpas* (powder mixtures) in which *Lauha bhasma* as major ingredients along with other herbal/mineral ingredients.[10] It was prepared by pure *Shilajit*, *Swarna makshik bhasma,* powder of rhizomes of *Shunthi* (*Zingiber officinale* Roscoe), fruits of *Marich* (*Piper nigrum* L.), and *Pippali* (*Piper longum* L) were taken in equal quantity in mortar and pestle, and 6 times of *Lauha bhasma* was added in it and triturated until it becomes fine powder and homogenously mixed.[6] The test drug was prepared in the Laboratory of Department of Rasa Shastra, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Drug treatments

All drugs were suspended in 0.3% w/v carboxymethylcellulose (CMC) for oral administration in animals. All doses were administered 1 h before the start of experiments. Applications volume in all cases was 10 ml/kg, and the control animals were treated accordingly with 10 ml/kg of 0.3% w/v CMC. Graded doses of SL were selected based on pilot study to establish dose-response relationship.[11]

Anti-diabetic activity

Type 2DM was induced in overnight-fasted rats by a single intraperitoneal (i.p.) injection of 65 mg/kg STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide.[12,13] Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72 h and then on the day 7 after injection.[14]

On the experimental day 1, all the rats were fasted overnight prior to nicotinamide-STZ treatment. Normal drinking water was provided. Immediately prior to injection, STZ was dissolved into 50 mM of sodium citrate buffer (pH 4.5) to a final concentration of 10 mg/ml. The STZ solution was freshly prepared for each rat and was injected within 5 min after being dissolved. STZ solution was injected intraperitoneally using 3 ml syringe and 23 gauge needle, at 65 mg/kg, 15 min after the i.p. administration of 120 mg/kg nicotinamide. Equal volume of citrate buffer (pH 4.5) was injected intraperitoneally for the control group. Rats were returned to their cages and were provide normal food and 10% sucrose water to minimize hypoglycemic shock. On the experimental day 2, 10% sucrose water was replaced with normal drinking water. On the experimental day 7, rats fasted overnight, and blood samples were collected. Blood glucose level was estimated. The animals with blood glucose concentration more than 200 mg/dl were used for the study.

The six groups used in this experiment were:

- Group I – Normal control (vehicle-treated)
- Group II – Diabetic control (vehicle-treated)
- Group III – Diabetic rats + SL 10 mg/kg/day
- Group IV – Diabetic rats + SL 30 mg/kg/day
- Group V – Diabetic rats + SL 100 mg/kg/day, and
- Group VI – Diabetic rats + glibenclamide 10 mg/kg/day.

Drug treatment was started on the 7th day after induction of diabetes (day 1 of the treatment). All the drugs were orally administered in the form of suspension in 0.3% CMC, once daily for 14 consecutive days. Body weight of rats was recorded periodically. Plasma glucose was measured on the 7th (day 1 of the treatment) and 14th day posttreatment. All other biochemical parameters were measured on the 14th day posttreatment.

Biochemical investigations

On the 14th day after overnight fasting, blood samples were collected from the retro-orbital venous plexus under light ether anesthesia using a glass capillary tube. Plasma was separated, and various biochemical parameters were analyzed as follows:

Estimation of blood glucose and plasma insulin

Glucose was estimated by glucose oxidase/peroxidase method using glucose estimation kit (Span Diagnostics Ltd., Surat, Gujarat, India).[13] Plasma insulin was measured by Enzyme Linked Immunosorbent Assay kit (DiaMetra Company, Segrate, Italy) using Bio-Rad micro-plate reader.[13]

Estimation of lipid profile

The levels of total cholesterol (TC), TGs, low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) were analyzed using a biochemical kit (Span Diagnostics Ltd., Surat, Gujarat, India).[15] The serum level of very LDL-C (VLDL-C) was calculated by using Friedewald formula.[16]

Statistical analysis

Data of all the experiments were expressed as mean ± standard error of mean of animals in each group (n = 6). Differences among different treatment groups were determined by one-way analysis of variance followed by Student–Newman–Keuls tests. GraphPad Prism (version 5.03, GraphPad Software, USA) was used for statistical analysis.

RESULTS

Effect on fasting blood glucose

Fasting blood glucose levels of animals challenged with nicotinamide-STZ were significantly higher as compared to the normal control animals ($P < 0.001$). In the diabetic control group, blood glucose level remained elevated until the 14th day of the experiment. Oral administration of SL (10, 30, 100 mg/kg/day) to diabetic rats significantly reduced fasting blood glucose as compared to vehicle treated diabetic control rats ($P < 0.001$) on the 14th day. On the last 14th day of the experiment, there was no statistically significant difference between the blood glucose levels of the SL 100 mg/kg treated diabetic group and the normal control group, such was also the case for the 10 mg/kg/day glibenclamide treated diabetic group. SL in 100 mg/kg dose almost normalizes the blood glucose level in 14 days of the study [Table 1].

Effect on plasma insulin

Plasma insulin level of rats subjected to nicotinamide-STZ challenge was significantly reduced ($P < 0.001$) compared to normal rats. All the doses of SL significantly increased plasma insulin level compared to diabetic control group ($P < 0.001$), but they did not normalize the reduced insulin level of diabetic rats in the treatment of 14 days. Glibenclamide treated diabetic rats also show a significant increase ($P < 0.001$) in plasma insulin level compared to vehicle treated diabetic control rats [Table 1].

Effect on lipid profile

On the 14th day of the study, nicotinamide-STZ challenged rats (diabetic control group) showed a significant elevation ($P < 0.001$) in plasma TC, TGs, LDL-C, and VLDL-C while the plasma HDL-C level decreased significantly ($P < 0.001$). Although the repeated dose administration of SL to diabetic rats significantly reversed, these changes to near normal levels ($P < 0.001$). The effect of SL (100 mg/kg) was comparable with that of glibenclamide at 10 mg/kg [Table 1].

DISCUSSION

Diabetes mellitus greatly influences utilization of carbohydrates, which in turn leads to imbalance in metabolism of lipids. Therefore, in management of diabetes, decreasing abnormally elevated blood glucose concentration to normal limits is of utmost importance. Among many forms of Type 2DM occurs predominantly and affects major population, i.e. 90% of diabetic patients. Alloxan and STZ both are widely used to induce experimental diabetes in animals. Alloxan causes rapid destruction of beta cells because of formation of superoxide radicals, and even in slight overdose, there is chances of higher mortality rate of animals because of renal failure, whereas STZ selectively destroys insulin-producing pancreatic beta cells by inducing DNA-strand break in these cells via glucose transporter 2 which seems more specific.[17] This causes activation of poly (ADP-ribose) polymerase, resulting in reduction of cellular NAD^+, and cell death. Nicotinamide partially protects the beta cells against the STZ mediated cytotoxic damage. Nicotinamide was found to preserve the

Table 1: Effect of *Shilajatvadi Lauha* on plasma glucose, serum insulin, and lipid profile of diabetic rats

Groups	Fasting blood glucose level (mg/dl)		Plasma insulin level (μIU/ml)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
	Day 1	Day 14						
1	94.16±3.52	95.16±2.35	18.33±0.48	79.5±0.76	51.16±0.61	40.5±0.76	28.36±0.96	10.66±0.48
2	266.00±3.28*	268.33±2.74	8.16±0.61*	141.67±0.88*	114.33±0.72*	24.5±0.77*	94.3±1.33*	22.86±0.14*
3	269.83±2.30	168.17±1.14¥	10.66±0.44¥	123.67±0.71¥	105.83±0.95¥	26.16±0.61¥	76.33±1.01¥	21.16±0.19¥
4	264.50±1.74	144.33±1.29¥	11.41±0.30¥	106.1±1.21¥	94.66±0.88¥	28.66±0.67¥	58.4±1.29¥	18.93±0.18¥
5	268.50±2.95	120.83±0.48¥	12.08±0.37¥	88.33±0.66¥	77.1±0.82¥	31.16±0.87¥	41.76±0.99¥	15.4±0.16¥
6	268.83±1.58	114.00±0.58¥	12.41±0.30¥	82.1±0.73¥	68.83±0.91¥	33.66±0.49¥	34.23±0.22¥	14.1±0.31¥

Value are mean±SEM, (n=6). *,¥Statistically significant difference relative to normal control (*P<0.001) and diabetic control (¥P<0.001), respectively. SEM: Standard error of mean, TC: Total cholesterol, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

intracellular pool of NAD^+ either by acting as a precursor of NAD^+ or by inhibiting the ADP-ribose polymerase.[18] Therefore, co-administration of nicotinamide (NA) and STZ produces stable moderate hyperglycemia suitable for chronic diabetes study, and it produces abnormal levels of blood glucose, plasma insulin, and serum metabolites such as cholesterol and TGs. Hence, these major parameters were taken into consideration while investigating hypoglycemic effect of SL.

Oral hypoglycemic agents and insulin have been used in the treatment of noninsulin and insulin dependent diabetes mellitus. While these drugs reduced blood sugar levels for 24 h and prevention of diabetic complications have not been so far achieved.[3] Long-term use of oral hypoglycemic agents can also have toxic effects on various organs of the body.[19]

Medicinal uses of SL for treatment of diabetes have been known to Indian systems of medicines since ancient times.[5,20,21] Several reports have also revealed broad spectrums of therapeutically interesting bioactivities of *Shilajit* compound formulation.[3,22] However, literature search did not reveal any systematic dose finding study necessary for properly defining the therapy relevant pharmacological activity profile of SL. Consequently, based on our pilot study to establish dose-response relationship, three graded doses, i.e. 10, 30, and 100 mg/kg/day were selected for anti-diabetic study of SL.[11]

Rats subjected to nicotinamide-STZ challenge showed significant increase in plasma glucose level. Two weeks repeated oral administration of SL and glibenclamide had resulted significant reduction in plasma glucose level of diabetic rats. It is well-established that glibenclamide produces hypoglycemia by increasing the secretion of insulin from the existing pancreatic β-cells.[23] In a study, treatment of moderate diabetic rats with some medicinal herbal drugs resulted in the stimulation of β-cells of islets of Langerhans, showing an insulinotropic effect.[24-26] In the present study, though all the doses of SL significantly lowered, the fasting blood glucose level and plasma insulin level in nicotinamide-STZ induced diabetic rats. These observations strongly suggest that like many therapeutically used anti-diabetic drugs;[24] stimulation of insulin increase from remnant beta cells is involved in the observed effects of SL in diabetic rats.

Among the various ingredients of herbo-mineral formulation SL have been reported for hypoglycemic effect; *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. It contains flavonoids and polyphenols which are natural antioxidants and significantly increases superoxide dismutase, glutathione, and catalase activities which are first-line defensive enzymes against free radicals.[27-30] *Rasayana* effect of *Lauha bhasma* reduces the degree of oxidative stress signaling pathways and, by that, preventing insulin resistance and β-cells dysfunction and, ultimately, controlling the blood sugar level, and its *Medohara* (hypolipidemic) effect decreases the high lipid level.[31] Copper (Cu) is one of the major constituents of *Swarna makshik bhasma* which is responsible for cholesterol and glucose metabolism in the body,[8,32] *Trikatu*; *Shunthi* (*Zingiber officinale* Roscoe), *Marich* (*Piper nigrum* L), and *Pippali* (*Piper longum* L) increase bioavailability by promoting rapid absorption from the gastrointestinal tract, or preventing metabolism/oxidation during the first passage through the liver after being absorbed, or a combination of these mechanisms, helping improve most drugs therapeutic activity, and it possess anti-glycation and antioxidants properties too which reduces the blood sugar level.[31,33,34] During preparation of *Lauha bhasma* (iron ash) huge amount of *Triphla*, *Haritaki* (*Terminalia chebula* Retz.), *Bibhitaki* (*Terminalia bellerica* [Gaertn.] Roxb.), and *Amalaki* (*Emblica officinalis* Gaertn) in equal proportions were used. It significantly reduced the blood sugar level in diabetic rats and decreased the effect of inflammatory

cytokine release in diabetics, which in turn might reduce the insulin resistance.[31] It possesses significant anti-diabetic and antioxidant property, because it increases superoxide dismutase, glutathione, and catalase activities in the blood and liver of experimental animals.[35-38] It has been shown that SL markedly improved the glucose tolerance and renormalizing the serum lipid and cholesterol level in nicotinamide-STZ induced diabetic rats; it may because of synergistic action of ingredients of SL which possess anti-diabetic, antioxidant, bioenhancer, rejuvenator, and free radical scavenging activity. It has been demonstrated through a validated and systematic study that traditionally used Ayurvedic formulation SL exerts significant anti-diabetic activity in rodents.

CONCLUSION

On the basis of the study, it can be concluded that *Shilajatvadi Lauha* has significant anti-diabetic activity in rats. It also showed good anti-hyperlipidemic property which can be attributed to synergistic effect of multiple herbo-mineral ingredients of formulation. Further studies should be undertaken to elucidate the mechanism of action of the observed anti-diabetic effect of *Shilajatvadi Lauha*, and clinical study is warranted for establishing therapeutic effectiveness.

Acknowledgment

Authors are thankful to Dr. Galib (Assistant Professor, Department of Rasa Shastra and Bhaishajya Kalpana, IPGT and RA, Gujarat Ayurved University, Jamnagar) and Dr. Ajit Kumar Thakur (Assistant Professor of Pharmacology, Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Noida) for his partial support in preparation of manuscript and experiments.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Erciyas F, Taneli F, Arslan B, Uslu Y. Glycemic control, oxidative stress, and lipid profile in children with type 1 diabetes mellitus. Arch Med Res 2004;35:134-40.
2. Holman RR, Turner RC. Oral agents and insulin in the treatment of non-insulin-dependent diabetes mellitus. Textbook of Diabetes. Oxford: Blackwell; 1991. p. 467-9.
3. Mitra SK, Gopumadhavan S, Muralidhar TS, Anturlikar SD, Sujatha MB. Effect of a herbomineral preparation D-400 in streptozotocin-induced diabetic rats. J Ethnopharmacol 1996;54:41-6.
4. Rasa Vagbhatta, Rasa Ratna Samuccaya, Commentary by Siddhinandan Mishra. Ver. 28/1. Reprint. Varanasi, India: Choukhambha Orientalia; 2011. p. 633.
5. Sharma H. Rasa Yoga Sagar. (Shakaradi Rasa). Vol. II, Ver. 413. Reprint. Varanasi, India: Chowkhambha Krishnadas Academy; 2004. p. 437.
6. The Ayurvedic Formulary of India. Lauha. Part-2. 2nd ed. New Delhi: Dept. of ISM and H, Ministry of Health and Family Welfare, Govt. of India; 2000. p. 311.
7. Bhattacharya SK. Shilajit attenuates streptozotocin induced diabetes mellitus and decreased in pancreatic islet superoxide dismutase activity in rats. Phytother Res 1995;9:41-4.
8. Singh N, Singh A, Chaudhary A. An experimental study of Swarna Makshik Bhasma as Antidiabetic medicine. Unique J Ayurvedic Herb Med 2014;2:1-6.
9. Johri RK, Zutshi U. An Ayurvedic formulation 'Trikatu' and its constituents. J Ethnopharmacol 1992;37:85-91.
10. Gupta KL, Pallavi G, Patgiri BJ, Galib, Prajapati PK. Critical review on the pharmaceutical vistas of Lauha Kalpas (Iron formulations). J Ayurveda Integr Med 2012;3:21-8.
11. Singh TR, Gupta LN, Singh RS, Kumar V, Kumar N. Comparative pharmaceutical and pharmacological evaluation of Shiljatvadi Lauha and Modified *Shilajatvadi Lauha*: A Preclinical study. MD Ayurveda thesis. Varanasi: Banaras Hindu University, Department of Rasa Shastra Institute of Medical Sciences; 2014. p. 162-84.
12. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, *et al.* Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998;47:224-9.
13. Husain GM, Singh PN, Singh RK, Kumar V. Antidiabetic activity of standardized extract of *Quassia amara* in nicotinamide-streptozotocin-induced diabetic rats. Phytother Res 2011;25:1806-12.
14. Wu KK, Huan Y. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol 2008;40:1-14.
15. Husain GM, Chatterjee SS, Singh PN, Kumar V. Hypolipidemic and antiobesity-like activity of standardised extract of *Hypericum perforatum* L. in Rats. ISRN Pharmacol 2011;2011:505247.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
17. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.
18. Bolzán AD, Bianchi MS. Genotoxicity of streptozotocin. Mutat Res 2002;512:121-34.
19. Holman RR, Turner RC, editors. Oral agents and insulin in the treatment of non insulin dependent diabetes mellitus. Oxford: Blackwell; 1991.
20. Shah NC. Bharat Bhaisajya Ratnakar. Part 5. Reprint edition. Rasaparakran. New Delhi: B Jain Publishers Pvt. Ltd.; 1999. p. 123.
21. Sharma SK. Rasakamdhenu of Chudamani Mishra, Second Part (4th Chikitsapad), Verse (kshaya roga) 33/264. 1st ed. Varanasi, India: Chaukhambha Orientalia; 1994. p. 72.
22. Adhikari KS, Ojha JK, Dwivedi KN. A clinical study of Shilajatu bijak yoga in Ojomeha pateints. J Res Ayurveda and Siddha 2003;24:62-70.
23. Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. Diabetes 2002;51 Suppl 3:S368-76.
24. Atta-Ur-Rahman, Zaman K. Medicinal plants with hypoglycemic activity. J Ethnopharmacol 1989;26:1-55.
25. Grover JK, Vats V, Rathi SS. Anti-hyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol 2000;73:461-70.
26. Nugroho AE, Lindawati NY, Herlyanti K, Widyastuti L, Pramono S. Anti-diabetic effect of a combination of andrographolide-enriched extract of *Andrographis paniculata* (Burm f.) Nees and asiaticoside-enriched extract of *Centella asiatica* L. in high fructose-fat fed rats. Indian J Exp Biol 2013;51:1101-8.
27. Surapaneni DK, Adapa SR, Preeti K, Teja GR, Veeraragavan M, Krishnamurthy S. Shilajit attenuates behavioral symptoms of chronic fatigue syndrome by modulating the hypothalamic-pituitary-adrenal

axis and mitochondrial bioenergetics in rats. J Ethnopharmacol 2012;143:91-9.
28. Wilson E, Rajamanickam GV, Dubey GP, Klose P, Musial F, Saha FJ, *et al.* Review on shilajit used in traditional Indian medicine. J Ethnopharmacol 2011;136:1-9.
29. Trivedi NA, Majumdar B, Bhatt JD, Hemavathi KG. Evaluation of Shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. Indian J Pharmacol 2004;36:373-6.
30. Shalam M, Harish MS, Farhana SA. Prevention of dexamethasone and fructose induced insulin resistance in rats by SH-01D, a herbal preparation. Indian J Pharmacol 2006;38:419-22.
31. Tanna I, Chandola HM, Joshi JR. Clinical efficacy of Mehamudgara vati in type 2 diabetes mellitus. Ayu 2011;32:30-9.
32. Banani D, Achintya M, Jayram H. Management of diabetes mellitus with current evidence and intervention with Ayurvedic rasausadhies. Indian J Tradit Knowl 2011;10:624-8.
33. Ramkisson JS, Mahamoodally MF, Ahmed N, Subratty AH. Antioxidant and anti-glycation activities correlates with phenolic composition of tropical medicinal herbs. Asian Pac J Trop Med 2013;6:561-9.
34. Atal CK, Zutshi U, Rao PG. Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. J Ethnopharmacol 1981;4:229-32.
35. Sasidharan I, Sundaresan A, Nisha VM, Kirishna MS, Raghu KG, Jayamurthy P. Inhibitory effect of *Terminalia chebula* Retz. fruit extracts on digestive enzyme related to diabetes and oxidative stress. J Enzyme Inhib Med Chem 2012;27:578-86.
36. Sabu MC, Kuttan R. Antidiabetic and antioxidant activity of *Terminalia belerica*. Roxb. Indian J Exp Biol 2009;47:270-5.
37. Nain P, Saini V, Sharma S, Nain J. Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. J Ethnopharmacol 2012;142:65-71.
38. Sharma RK, Patki PS. Double-blind, placebo-controlled clinical evaluation of an Ayurvedic formulation (GlucoCare capsules) in non-insulin dependent diabetes mellitus. J Ayurveda Integr Med 2010;1:45-51.