Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

ANTIDIABETIC ACTIVITY OF SWARNA MAKSHIKA BHASMA, SHUDDHA SHILAJATU AND THEIR COMBINATION IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

Singh Sanjeev [1]*, Sharma Khemchand [2]

[1] Assistant Professor, Department of Rasashastra Evam Bhaishajya Kalpana, Jeevan Jyoti Ayurvedic Medical College & Hospital, Aligarh, Uttar Pradesh, India

[2] Head & Professor, Department of Rasashastra Evam Bhaishajya Kalpana, Rishikul Government P.G. Ayurvedic College & Hospital, Haridwar, Uttarakhand, India

Received on: 30/11/21 Accepted on: 15/01/22

***Corresponding author**
E-mail: drsanjeev.8791@gmail.com

DOI: 10.7897/2277-4343.13017

ABSTRACT

Swarna makshika bhasma and Shilajatu are mentioned by Acharya Sushruta in Madhumeha Chikitsa. These two Rasayanas play a role in glucose and insulin metabolism. The present study was undertaken to assess the anti-diabetic activity in Wistar strain albino rats, thirty-six in number divided into six groups. Streptozotocin was given to induce diabetes. Glibenclamide was used as a standard control drug. Doses of all drugs in each group were according to an accepted standard. Mild diabetic rats (blood sugar > 200 mg/dL) were given medication after their body weight and blood glucose levels were determined. The treatment was maintained on days 7, 14, 21, and 28, and biochemical parameters were calculated. From the fourth day of Streptozotocin induction of diabetes, Makshika bhasma, Shuddha shilajatu and their combination, Glibenclamide, were given once daily. After administration, the effects of Swarna makshika bhasma, Shuddha shilajatu, and Combination of Makshika bhasma and Shuddha shilajatu on bodyweight were statistically non-significant. However, there was a distinction between the before and after-effects. The most remarkable changes in blood glucose levels were reported for Makshika bhasma, Shuddha shilajatu, and Makshika bhasma with Shuddha shilajatu. To conclude, the Combination of Shilajatu and Makshika bhasma is the most efficient and remarkably therapy alternative in Madhumeha. Their multifaceted impact makes them a viable method in the long-term control of diabetes.

Keywords: Swarna Makshika, Shuddha Shilajatu, Glibenclamide, Madhumeha, Anti-diabetic

INTRODUCTION

In Ayurveda, diabetes mellitus (DM) is referred to as Madhumeha. Diabetes is one of the most common metabolic non-communicable endocrine illnesses[1]. Ayurvedic principles are based on clinical observations that have been tested over time. These concepts of scientific logic are founded not just on ancient sources but also on their ability to be demonstrated[2]. Ayurveda prescribes many herbs, polyherbal formulations, herbo-mineral preparations, and therapeutic techniques to prevent, relieve, and control diabetes mellitus. Metal imbalances are critical in disrupting average glucose and insulin metabolism in diabetics. Changes in metal status can also enhance oxidative stress, leading to insulin resistance and diabetic complications[3]. In Madhumeha Chikitsa, Acharya Sushruta only mentioned three Rasayana i.e. Shilajatu, Makshika, and Tuvaraka. Shilajatu is well-known for its Naimittika Rasayana impact, as well as it's Oja vardhaka and Pramehagna qualities. Swarna makshika bhasma is a Rasayana known for its Dehavada and Lohavada properties. They function primarily at the rasa dhatu (increases nutritional status), improve the quality of dhatu production by improving Agni and eliminating Srothorodha, and improve the quality of Dhatu production by improving Agni and removing Srothorodha. In the end, it may reduce Dhathu shaithilya while strengthening the Ojas[4].

Rasaushadhis are utilised as a rapid saviour of diseases in Ayurvedic medicine. Modern science prefers replicable and revalidated facts to accept any molecule/substance as medication. As a result, we planned and carried out this study on streptozotocin-induced diabetic rats to gather data on the efficacy and safety of Makshika bhasma and Shuddha shilajatu and their combination in the treatment of diabetes.

MATERIALS AND METHODS

For this study, Wistar strain albino rats (both male and female) weighing 138-186 gm. were obtained from the Animal house of Datta Meghe Institute of Medical Sciences (DU.), Wardha (Maharashtra). The animals were given a two-week acclimatisation period. They were fed a balanced diet with black gram, mat bean, wheat flour, salts, oil and vitamins mixture, etc. The amount of pure water was given daily. Rats were kept in a well-ventilated chamber with a constant temperature of 28±5°C. They were kept in polypropylene cages with paddy husk beds that were replaced every 4-5 days and were exposed to natural light and dark cycles (12:12). The animals are randomly selected for proper identification marked with Picric acid (used as a dye). The experimental protocols were approved by Institutional Animal Ethics Committee (DMIMS (DU) /IAEC/2016-17/12) dated 12/09/2018, following the guideline formulated by CPCSE, India.

Drugs and Chemicals

1. Test Drugs:
(i) Swarna Makshika Bhasma (SMB)
(ii) Suddha Shilajatu (SS)
(iii) Combination of Swarna Makshika Bhasma and Suddha Shilajatu (SMB + SS)

These drugs were prepared in P.G. Department of Rasashastra & Bhaishajya Kalpana, Rishikul Campus, Haridwar (Uttarakhand).

2. Standard Drug: Glibenclamide procured from Central Research Laboratory (CRL) of DMIMS (DU), Wardha (Maharashtra).
3. Vehicle: Normal Saline
4. Diabetes Inducing Agent: Streptozotocin of Himedia laboratories [Item Code-CMS1758-250MG] was procured from Genex Scientific, Nagpur (Maharashtra).
5. Citric Acid and Tri-Sodium Citrate: Procured from Genex Scientific, Nagpur (Maharashtra). To prepare buffer solution (pH - 4.5) to prepare Streptozotocin (STZ) injection.

Diabetes Induction

A1ml intraperitoneal injection of streptozotocin (40 mg/Kg) in 1 M Citrate buffer (made by dissolving 105.07mg citric acid in 50ml distilled water and 147.05mg sodium citrate in 50ml distilled water and combining the two solutions to form 100ml of citrate buffer) was given. Rats with hyperglycaemia (blood glucose levels of 200-300 mg/dl) were used in the experiment after 48 hours of STZ treatment. The drugs were given out for 28 days. The medicine was administered one hour before the investigation on the seventh day, and blood samples were taken from the rat's retro-orbital plexus. Serum was separated by centrifugation at 3000rpm for three minutes, and biochemical parameters were calculated 7, 14, 21, 28 Days.

Experiment Schedule & Treatment Protocol

In the present study, 36 albino rats were divided into six groups, with six rats in each group.

Dose Fixation

1. Dose of Streptozotocin: The dose of streptozotocin for diabetes induction was fixed as 40mg/kg body weight via the intraperitoneal (I.P) route.
2. Dose of Glibenclamide: The dose of the standard drug Glibenclamide was fixed as 0.45 mg/kg body weight orally once daily for 28 days.
3. Dose of Swarna Makshika Bhasma: Human dose of Swarna makshika bhasma mentioned in Rasatarangini is ½ to 2 Ratti.
4. Dose of Suddha Shilajatu: Human dose of Suddha shilajatu mentioned in Rasatarangini is 2 to 8 Ratti.

Test Drug Dose Calculation: Dose fixation of test drugs for rats was calculated on the base of body surface area ratio (conversion factor 0.018 for rat and 0.0026 for mice), known as Paget & Barnes formula (1964).

Therapeutic Dose

1. Dose of Swarna Makshika Bhasma (SMB) is 250 mg.
2. Dose Suddha Shilajatu (SS) is 1 gm.
3. Dose of Combination of Swarna Makshika Bhasma & Suddha Shilajatu

(SMB & SS) = 1250mg [250 mg + 1 gm]

Test drug dose Calculation

Human dose X body surface area ratio conversion factor = 250 mg x 0.018 = 4.5 mg /200 gm. of rat.

For converting, gm. to kg. this dose is multiplied by suitable factor 5

Finally, converting Dose – 22.5 mg/kg. (For Swarna Makshika Bhasma)

Same dose calculation method applied for Shilajatu, Combination (SMB + SS) and Standard drug. The details are in table 1.

On biochemical markers such as blood glucose, the effects of Makshika bhasma, Shuddha Shilajatu, and the conventional medication Glibenclamide were detected. The entire experiment took 28 days to complete. On the first day, streptozotocin was given. Day 1 (start of treatment) was three days after the glucose level was measured, and mild diabetic (blood sugar > 200 mg/dL) rats were started on treatment. Treatment was continued, and biochemical parameters were measured on days 7, 14, 21, and 28. Blood was drawn by puncturing retro-orbital plexuses and then centrifuged at 3000 rpm for 20 minutes. The results were analysed using a paired t-test for intergroup comparison and a one-way ANOVA for intergroup comparison, $p<0.05$ values were deemed statistically significant.

RESULTS AND DISCUSSION

Adult rats of either sex were given intraperitoneal streptozotocin (STZ, 40 mg/kg stat) to induce moderate diabetes. Before the experiments, the rats fasted for 18 hours. Intergroup comparison on mean body weight can be observed in figure 1, and intragroup comparison on mean body weight can be observed in figure 2. Intergroup comparison on mean blood glucose can be observed in figure 3, and intragroup comparison on mean blood glucose can be observed in figure 4. Comparative analysis of Intragroup (within-group comparison) for the mean difference in body weight can be observed in table 2. Comparative analysis of Intragroup (within-group comparison) for the mean difference in blood glucose can be observed in table 3. On body weight and blood glucose, the effects of Swarna makshika bhasma, Shilajatu and the conventional medication Glibenclamide were identified. Three days after streptozotocin induction, body weight and blood glucose levels were measured, and mild diabetic rats (blood sugar > 200 mg/dL) were given therapy. On days 7, 14, 21, and 28, the treatment was continued, and biochemical parameters were estimated. Adult rats of either sex were given intraperitoneal streptozotocin (STZ, 40 mg/kg stat) to induce moderate diabetes. Makshika bhasma, Shuddha Shilajatu and Glibenclamide was administered once daily from the 4th day of STZ. Effects on body weight for Makshika bhasma, Shuddha Shilajatu and their combination were statistically non-significant after administration. But there was a difference in before and after-effects. In the blood glucose level for Makshika bhasma, Shuddha Shilajatu and their combination, primarily significant changes were observed.

After diabetes induction, using streptozotocin (STZ) injection at the rate of 40 mg/kg body weight in groups 2, 3, 4, 5 and 6 statistically significant increase in blood sugar level and decrease in body weight. After that, no intervention was given in group 2; Glibenclamide was administered in a dose of 0.45 mg/kg body weight in group 6. Intergroup comparison was made by Tukey multiple comparison test whereby all the groups viz. normal control, test drug Samples and standard drug samples were compared with groups. Group 1 (Control group) has no changes in FBSL observed after 28 days oral on administration normal saline (5ml/kg body weight.). In Group 2 (Disease control group), finding suggested that blood sugar levels increased in this group. No drug was administered during this period (28 days). In Group 3, Makshika bhasma was administered at a 22.5 mg/kg body weight test sample dose. In Group- 4 (Suddha Shilajatu), at test sample dose of 90 mg/kg body weight and group 5 at test sample dose 112.5mg/kg found that Combination (SMB & SS) drug was statistically more significant in comparison of Makshika bhasma and Suddha Shilajatu. In group-6, on administered Standard drug Glibenclamide at a dose of 0.45 mg/kg body weight compared with Group 3, Group 4 and Group 5 found statistically more significant.

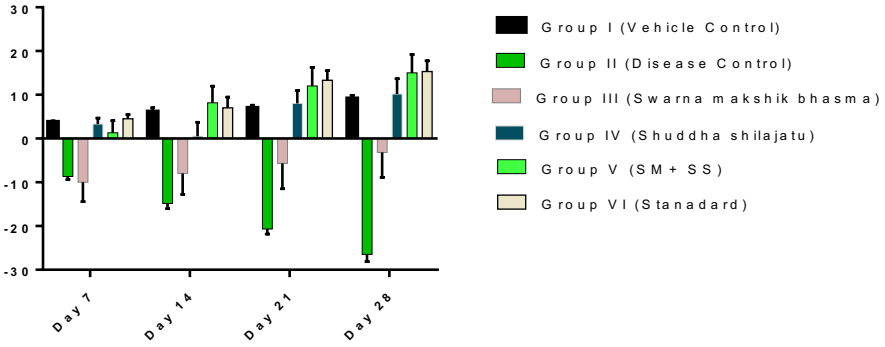

Figure 1: Comparative data (inter-group) on mean body weight

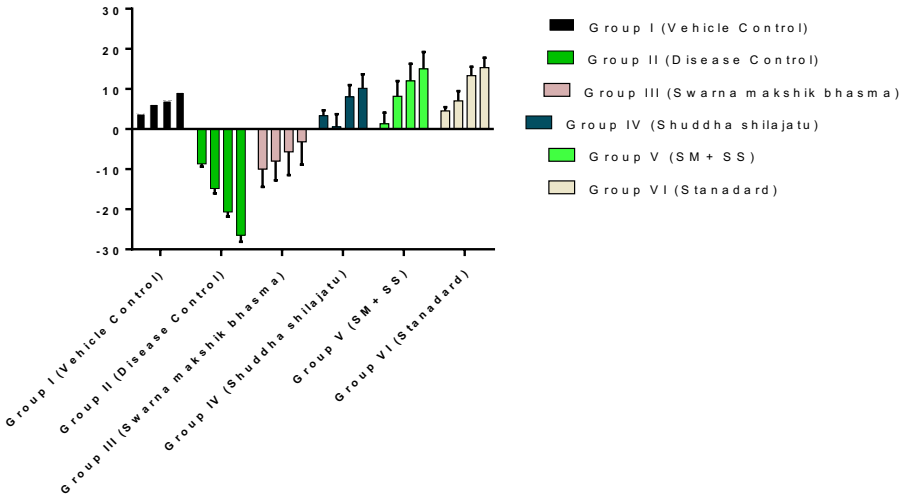

Figure 2: Comparative data (intra-group) on mean body weight

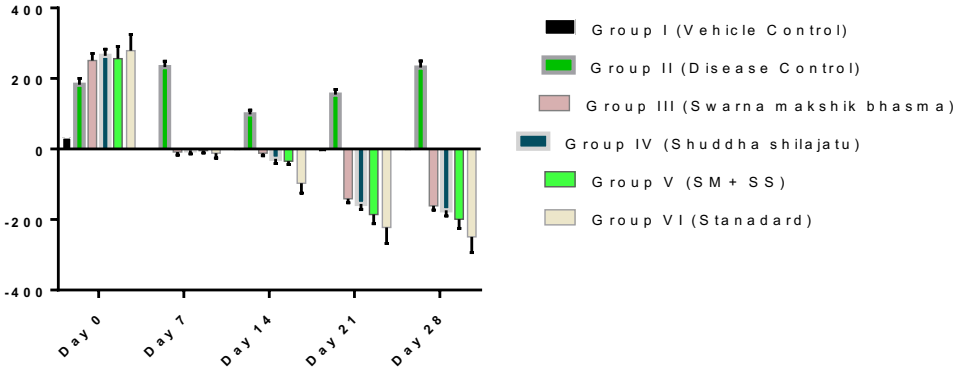

Figure 3: Comparative data (inter-group) on mean blood glucose

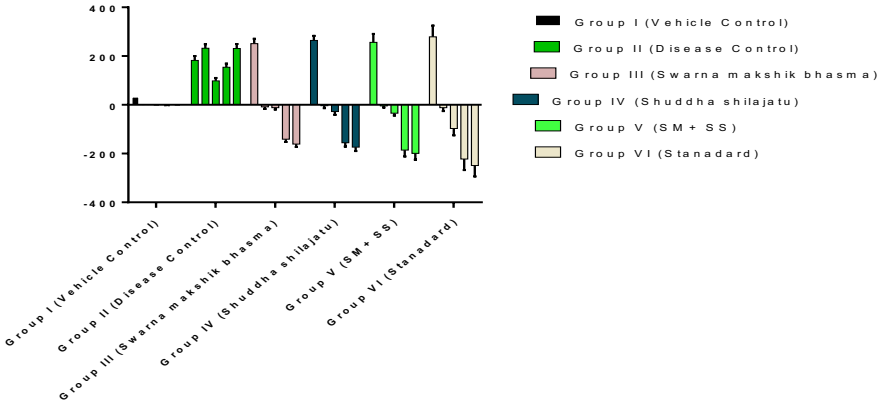

Figure 4: Comparative data (intra-group) on mean blood glucose

Table 1: Detailed description of groups, formulation and dose

Animal Groups	Number of rats	Group Name	Formulation and Dose
Group I	6	Normal Control (5ml/kg)	Normal Control (5ml/kg) orally
Group II	6	Diabetes Induced Control (Received normal saline 5ml/kg)	Received normal saline 5ml/kg) orally
Group III	6	Diabetes induced test sample I (Swarna Makshika Bhasma)	Swarna Makshika Bhasma 22.5 mg/kg orally
Group IV	6	Diabetes induced test sample II (Suddha Shilajatu)	Suddha Shilajatu 90 mg/kg orally
Group V	6	Diabetes induced test sample III (Swarna Makshika + Suddha Shilajatu) Combination	Combination (Swarna Makshika Bhasma 22.5 mg/kg + Suddha Shilajatu 90 mg/kg) orally
Group VI	6	Diabetes induced Standard drug (Glibenclamide)	Standard Drug Glibenclamide 0.45 mg/kg orally

Table 2: Comparative analysis of Intragroup (within-group comparison) for the mean difference in body weight

	Group I (Vehicle Control)	P-Value	Group II (Disease Control)	P-Value	Group III (Swarna Makshik hasma)	P-Value	Group IV (Shuddha shilajatu)	P-Value	Group V (SM+ SS)	P-Value	Group VI (Standard)	P-Value
Day 7 vs Day 14	-2.333	0.9494	6.167	0.4872	-2	0.9672	2.833	0.9139	-6.833	0.3953	-2.5	0.9388
Day 7 vs Day 21	-3.167	0.8845	12	0.0325	-4.333	0.7495	-4.667	0.7040	-10.67	0.0712	-8.833	0.1795
Day 7 vs Day 28	-5.333	0.6083	17.83	0.0004	-6.833	0.3953	-6.833	0.3953	-13.67	0.0108	-10.83	0.0649
Day 14 vs Day 21	-0.8333	0.9975	5.833	0.5353	-2.333	0.9494	-7.5	0.3121	-3.833	0.8127	-6.333	0.4636
Day 14 vs Day 28	-3	0.8998	11.67	0.0399	-4.833	0.6806	-9.667	0.1206	-6.833	0.3953	-8.333	0.2236
Day 21 vs. Day 28	-2.167	0.9589	5.833	0.5353	-2.5	0.9388	-2.167	0.9589	-3	0.8998	-2	0.9672

Table 3: Comparative analysis of Intragroup (within-group comparison) for the mean difference in blood glucose

	Group I (Vehicle Control)	P-Value	Group II (Disease Control)	P-Value	Group III (Swarna makshik bhasma)	P-Value	Group IV (Shuddha shilajatu)	P-Value	Group V (SM+ SS)	P-Value	Group VI (Standard)	P-Value
Day 0 vs Day 7	34	0.7820	-49.67	0.4535	259.3	<0.0001	267.8	<0.0001	260.3	<0.0001	290.5	<0.0001
Day 0 vs. Day 14	37.17	0.7207	83.83	0.0422	262.5	<0.0001	292	<0.0001	290.5	<0.0001	375.7	<0.0001
Day 0 vs. Day 21	40.33	0.6548	27.83	0.8817	392.2	<0.0001	420	<0.0001	441.7	<0.0001	501.2	<0.0001
Day 0 vs. Day 28	36.5	0.7340	-48.5	0.4781	411.5	<0.0001	437.7	<0.0001	454.8	<0.0001	527.8	<0.0001
Day 7 vs Day 14	3.167	>0.9999	133.5	0.0001	3.167	>0.9999	24.17	0.9259	30.17	0.8475	85.17	0.0374
Day 7 vs Day 21	6.333	0.9995	77.5	0.0734	132.8	0.0001	152.2	<0.0001	181.3	<0.0001	210.7	<0.0001
Day 7 vs. Day 28	2.5	>0.9999	1.167	>0.9999	152.2	<0.0001	169.8	<0.0001	194.5	<0.0001	237.3	<0.0001
Day 14 vs. Day 21	3.167	>0.9999	-56	0.3289	129.7	0.0002	128	0.0003	151.2	<0.0001	125.5	0.0004
Day 14 vs Day 28	-0.6667	>0.9999	-132.3	0.0002	149	<0.0001	145.7	<0.0001	164.3	<0.0001	152.2	<0.0001
Day 21 vs. Day 28	-3.833	>0.9999	-76.33	0.0809	19.33	0.9661	17.67	0.9756	13.17	0.9919	26.67	0.8971

In Ayurvedic medicine, mineral medications are always better than herbal and animal items. Mineral preparations are chosen over herbal formulations because they have better properties such as quick action, palatability, effectiveness with low doses, and a long shelf life[3]. Makshika bhasma and Shilajatu are micronutrient-rich minerals that, with proper processing, become highly effective and can be used to treat ailments such as Prameha[4]. The body comprises more than seventy elements, and a lack of these critical elements can lead to various problems[5]. Diabetes mellitus can cause trace element homeostasis to be disrupted. In diabetes mellitus, however, a change in trace element status may lead to insulin resistance and the development of diabetic complications[6]. The most effective strategy to prevent or reduce these consequences is maintaining strict glycemic control. This is easily accomplished with the help of a few minerals and vitamins that can also act as antioxidants, lowering diabetes complications.

Shilajatu comprises humic compounds such as DBP, Fulvic acid, and Humic acid. It also contains micronutrients such as Fe, Zn, Mn, Mg, V, K, Zn, Ni, and various chemical compounds such as benzoic acid and amino acids. Shilajatu's immunomodulatory action has inhibited macrophage and lymphocyte activation and migration. Furthermore, as an antioxidant, it will protect the pancreatic islet cell from cytotoxic oxygen radical damage[7,8,9]. Long-term Shilajit medication increases the number of beta cells in the pancreas, a process known as pancreatotrophic activity, leading to improved pancreatic sensitivity and fast production of a significant amount of insulin in response to hyperglycaemia. Shilajatu's hypoglycaemic impact (1000 mg/kg) is much more than metformin's (500 mg/kg)[10].

Copper, iron, and sulphur, the primary three components of Makshika bhasma, are vital and play a role in synthesising haemoglobin. XRD analysis of several Makshika bhasma samples revealed that raw Swarna makshika includes $CuFeS_2$, which following Shodhana was transformed into copper and iron sulphides, oxide, and sulphate of iron. Fe_3O_4, Fe_2O_3, FeS_2, $FeSO_4$, and Cu_2S were the most common chemicals in Bhasma samples[11]. Mg, K, Si, and Al were discovered and trace elements[12].

CONCLUSION

Regulation of blood glucose levels in diabetes can prevent the various complication associated with the disease[13]. Metal aberrations disrupt average glucose and insulin metabolism in diabetics, and changes in metal status might increase oxidative stress, which contributes to diabetic complications. These minerals are high in micronutrients, making them more beneficial. The goal of diabetes treatment is to avoid both acute and long-term microvascular and macrovascular problems[14]. The effectiveness of Makshika bhasma and Suddha Shilajatu was very much prominent. Combination (SMB & SS) drug is less significant than Standard drug but more significant than Makshika bhasma and Suddha Shilajatu as observed in our study. These Rasayanas can help with glycemic control as well as lipid profile improvement. They are adequate for curative and preventative purposes, provide a robust and healthy body, and delay ageing. As a result, we may conclude that Shilajatu and Makshika bhasma rasayanas are two of the best accessible therapy options in Madumeha. Their various action makes them a promising approach in the long-term management of diabetes mellitus.

REFERENCES

1. Banerjee S, Debnath P, Rao PN, Tripathy TB, Adhikari A, Debnath PK. Ayurveda in changing scenario of diabetes management for developing safe and effective treatment choices for the future. J Complement Integr Med. 2015;12(2):101-10.
2. Valiathan MS. Towards Ayurvedic Biology; A Decadal Vision Document-2006. Bangalore: Indian Academy of Sciences; 2006
3. Bonnefont-Rousselot D. The role of antioxidant micronutrients in preventing diabetic complications, Treat Endocrinol, 2004:3(1); 41-52.
4. Chandran S, Patgiri BJ, Dharmarajan P. Shilajatu and Swarna Maakshika– A promising ayurvedic combination in the management of Madumeha (Diabetes). Journal of Ayurvedic and Herbal Medicine 2016; 2(3): 96-99.
5. Gupta RK, Lakshmi V, Mahapatra S, Jha C B. Therapeutic uses of *Swarnamakshika bhasma* (A critical review). AYU 2010; 31:106-10.
6. Basaki M, Saeb M, Nazifi S, Shamsaei HA. Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. Biol Trace Elem Res. 2012;148(2):161-4.
7. Bhattacharya SK. Shilajit attenuates streptozotocin-induced DM & decrease pancreatic islet superoxide dismutase activity in rats. Phytotherapy Research 1995;9 (1):41-44.
8. Bhattacharya SK, Sen AP. Effect of shilajit on biogenic free radicals. Phytotherapy Research1995; 9:56-59.
9. Ghosal S, Soumya L, Kumar Y. Interaction of Shilajit with biogenic free radicals. Indian J Chem 1995;34B:596-602.
10. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. Indian journal of pharmacology 2004;36(6):373-376.
11. Gupta RK, Lakshmi V, Jha CB. X-Ray Diffraction of different samples of Swarna Makshika Bhasma. AYU 2015; 36 (2):225-9.
12. Mohapatra S, Jha CB. Analytical study of raw Swarna Makshika (Chalcopyrite) and its Bhasma through TEM and EDAX. AYU 2013;34(2):204-208.
13. Gupta Satish Chand et al. Evaluation of Antidiabetic Activity of Methanolic Extracts of Root And Stem of Gmelina Arboria Roxb. In Streptozocin Induced Diabetic Rats. Int. J. Res. Ayurveda Pharm 2015; 6 (3):325-334.
14. Sharma RK, Patki PS. Double-blind, placebo-controlled clinical evaluation of an Ayurvedic formulation (Gluco Care capsules) in non-insulin-dependent diabetes mellitus. J Ayurveda Integr Med. 2010;1(1):45-51.

Cite this article as:

Singh Sanjeev and Sharma Khemchand. Antidiabetic activity of swarna makshika bhasma, shuddha shilajatu and their combination in streptozotocin-induced diabetic wistar rats. Int. J. Res. Ayurveda Pharm. 2022;13(1):27-31 http://dx.doi.org/10.7897/2277-4343.13017

Source of support: Nil, Conflict of interest: None Declared