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THE EXPERIMENTAL STUDY OF SHILAJATU ON ALLOXANISED ALBINO RATS ON DIABETIES MELLITUS

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ABSTRACT

The claims of anti diabetic effect of metals and minerals made in Indian system of medicine are mainly based on clinical observation. But present day trend demands scientific research, to replace the empericalism by rationalism .so in order to verify the age old claims, scientific experimentation using animal models is primafacie necessity and the claims should also be supported by clinically research for a valid conclusion. *Shilajatu* is in use for clinical condition including Diabetes mellitus, but the reported literature does not reveal any scientific basis of its clinical use in diabetic patient. Hence the present study was undertaken to rationalize the reported use of *Shilajatu* in Indian system of medicine.

KEYWORDS: Diabetes, Experimental Study, *Shilajatu.*

INTRODUCTIONS

Experimental models are the tools of research, hence the present study was undertaken in aniamals to evaluate the possible antidiabetic effect of shilajatu an attempt was also made to find out the possible mechanism of anti diabetic effect of *shilajatu.*

The experimental study was conducted at Dept. of Pharmacology and Dept. Of Pathology, IMS, BHU, Varanasi India on albino rats both for blood glucose estimation and histopathological study.

The following experiments were designed for the present study

- To study the blood glucose level and beta-cells regeneration within 4 weeks of Alloxan administration.
- To study the effect of *shilajatu* on Alloxan induced hyperglycaemia in albino rats.
- To study the effect of *shilajatu* on Beta-cells regeneration.

MATERIAL AND METHODS

1. Animals

Inbreed (Fischer-Strain) albino rats weighing between 100-150 gm of either sex obtained from the central animal house of IMS, BHU, Varanasi India were used. Animals were housed in colony cages in temperature controlled (25±2°C) animal rooms with light and dark cycles of 12 hrs respectively and relative humidity 55-60% for at least one week before the experiment. The rats were fed Hind lever pellets.

2. Drugs

- *Shilajatu*—obtained from the market of Nepal. Fresh suspension of shilajatu was made prior to experimentation using distilled water.
- Insulin—(Soluble) 40 units/UI (Boots India)
- Alloxon Monohydrate (B.D.H. England)
- Chemicals

a. Glucose (S.D. Fine Chemicals, Bombay India)
b. Sodium Fluoride -do-
c. Potassium Oxalate -do-
d. GOD/POD kit (Span Diagnostic P.V. Ltd Udhan Surat, India) Consist of
Reagent 1: Glucose Enzyme reagent

Reagent 2: Glucose standard 100%

Reagent 3: Phenol reagent.

- **Instruments**

a. Photoelectric calorimeter type III (Systronics, Naroda Industrial Area Ahmedabad, India)
b. Centrifuse
c. Micropipette
d. Glasswares. All glass wares were of corning quality.

Following steps were observed for making albino rats hyperglycaemic

a. Rats were fasted for 24 hrs with water given ad libitum.
b. Insulin (soluble) injected subcutaneously in the dose of 1 unit/kg body wt. in fasted rats.
c. One hour after injecting insulin, solution of alloxan monohydrate in distilled water was injected intraperitoneally in the dose of 150 mg/kg body wt. then measured food (150gm/100gm wt) was given.

Alloxan produces a triphasic blood sugar response i.e. Early Hyperglycaemia, Hypoglycaemia and Late hyperglycaemia.

These were confirmed by the electron microscopic studies. The changes observed were vaculation of mitochondria, fragmentation of nuclear and plasma membranes followed by disintegration of cell. The degenerative changes were not observed in Alpha-cells.

Procedure

Blood sugar was estimated and animals having blood sugar above 200mg were taken. All the animals were frank diabetes. The animals were divided into four groups i.e.

- Control gr.—only equal volume of distilled water of eight animals
- Group l -- Dose of 200 mg./kg/day body wt *shilajatu* of eight animals
- Group ll-- Dose of 500 mg./kg/day body wt *shilajatu* of eight animals
- Group lll-- Dose of 1000 mg./kg/day body wt *shilajatu* of nine animals.

After estimation of 2nd day blood sugar, every 7th day blood sugar was estimated and continued up to 28th day i.e. 7th, 14th, 21st, and 28th day.

In control gr. Only five animals were alive at the end of experiment. In treated l and ll gr. six animals were alive at the end of experiment. In gr.lll only one animal died after 7th day in remaining were alive till the completion of experiment.

For biochemical study we adopted GOD/POD method for Blood sugar estimation. It is an invitro enzymatic calorimetric method for the quantitative determination of glucose in serum/plasma. It is very simple, convenient and highly economic.

HISTOPATHOLOGICAL STUDIES

1. Beta cell regeneration during four weeks of Alloxan administration-

12 albino rats of either sex weighing 100-150 gms were taken and kept under fasting for 24 hrs .the diabetes was induced by the method as described earlier .

Blood glucose level was estimated and the animals having blood sugar above 200 mg % were taken. All the animals were frank diabetic. They were divided into 4 group i.e. three animals in each group. Blood sugar was estimated on 7th, 14th, 21st and 28th day. Animals of 1 st group were sacrificed after one week and second group in 2nd week in the same way 3rd and 4th group animals were sacrificed in 3rd and 4th week respectively.

2. Effect of *Shilajatu* on Beta- cells

All the three groups of hyperglycemic animals which were treated with *shilajatu* were sacrificed on 28th day and their pancreas were preserved in 10 % formalin solution for histopathological studies of Beta -cells. The findings were compared with that of controlled.

After taking out retroperitoneal fat containing pancreatic tissue from the albino rat, dehydration was done in graded % of alcohol from 70% to 100%. Following their dehydration multiple sections from the site of the tissue were taken and processed to prepare paraffin embedded tissue blocks. Form each block two section were taken and one is stained with H and E (Haematoxyline and Eosin) and other was stained with Gomoris Aldehyde Fuchin stain (Culling, 1963) and shape and size of Islets of langerhans and population of beta-cells were studied. The beta cell population was quantitated bycounting the beta cells.

RESULTS

1. *Shilajatu*-Alloxan Induced Hyperglycaemia (A.I.H)

In A.I.H experiment, were diabetes was produced by Alloxan, the mean blood sugar in control group shows rise on 14th day but decrease was observed on 21st day and again rise in blood suger level was observed on 28th day.

Regular fall in blood suger level was observed in all the treated group but statistically data was insignificant in the treated group 2nd (200 mg/kg) and 3rd(500 mg/kg) up to 7th day but after that from 14th day to 28th day reading were statistically significant. In treated group lll regular fall in blood sugar level was observed from 7th day to 28th day and was found statistically significant.

2. Beta-cell Regeneration within four weeks of Alloxan Administration

In this experiment all the alloxanised rats were frank diabetic. It is evident from the Table-2 that there was regular rise in blood sugar upto the 28th day. The normal beta-cell number was markedly reduced after alloxan administration. Massive necrosis and shrinkage of beta-cells were also seen after 7 day of alloxan administration and this nacrosis persists upto 28th days. Hence these findings confirm the concept of selective necrosis of beta-cells and permanent diabetes after alloxan administration.

3. Effect of *shilajatu* on beta-cell regeneration

It was evident from the histopathological study that-

- In control group out of five animals three of them showed necrosis of islet cells and reduction in the

number of granules but two animals showed slight regeneration of beta-cell after 28th day.

- In treated group l (200 mg/kg body wt.) out of six animals four animal showed massive necrosis of beta-cells while in one animal slight regeneration of beta-cells was observed and one of the animal of this group showed increase in number as well as size of beta-cells.
- In treated group ll (500mg/kg body wt.) out of six animals three of them showed necrosis and decrease cellularity of beta-cells while rest of animals showed spontaneous regeneration with increase in number as well as size of the beta-cell but this was not marked.
- In treated group lll (1000mg/kg body wt.) poor regeneration of beta-cell was observed in all the animals.

Table 1: Effect of Alloxan on Blood Glucose Level in Rats (28 days).

	BLOOD GLUCOSE LEVEL					
Groups	**Fasting blood sugar**	**48 hours**	**7 days**	**14 days**	**21 days**	**28 days**
Control	82.33±1.45 A	236.67±8.82* A1	246.67±10.14 A2	-	-	-
l	77.67±1.45 B	202.5±10.61* B1	225.0±8.66 B2	232.33±8.68 B3	-	-
ll	85.0±7.63 C	243.17±11.80* C1	246.67±13.0 C2	250.0±11.55 C3	253.33±13.02 C4	-
lll	85.0±2.89 D	225.67±17.90* D1	241.67±14.81 D2	244.0±14 .0 D3	246.33±10.17 D4	248.33±11.67 D5

Animals of Control Group, l, ll, and lll were sacrificed after 7th , 14th , 21st and 28 days respectively
* -- P‹ 0.005

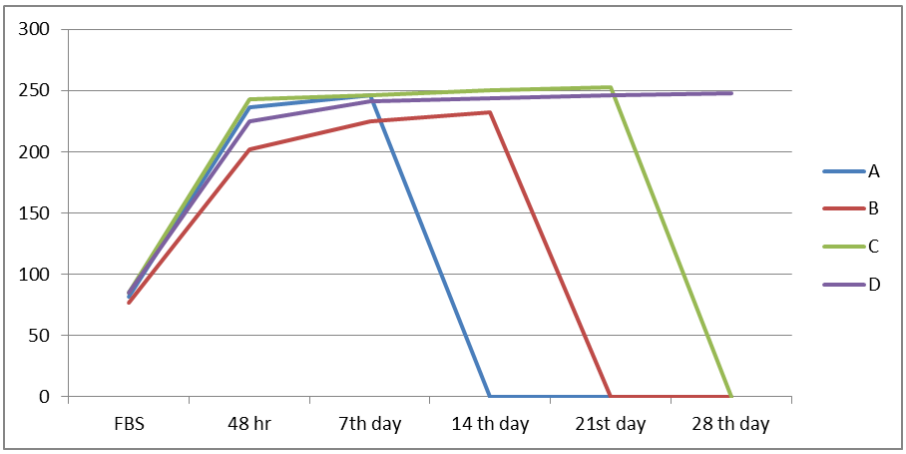
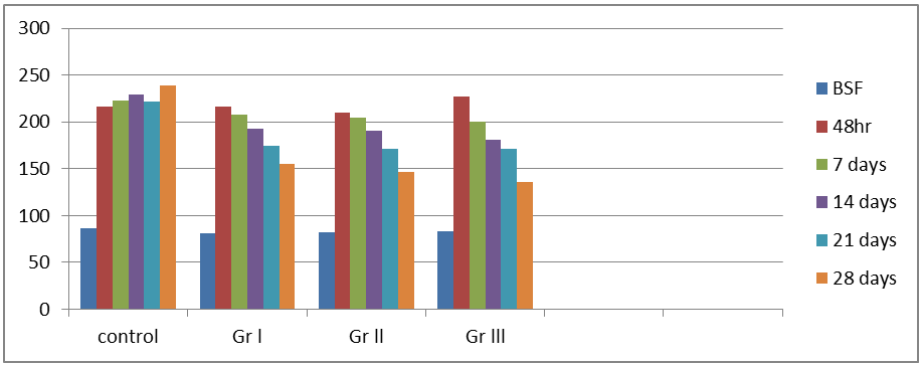
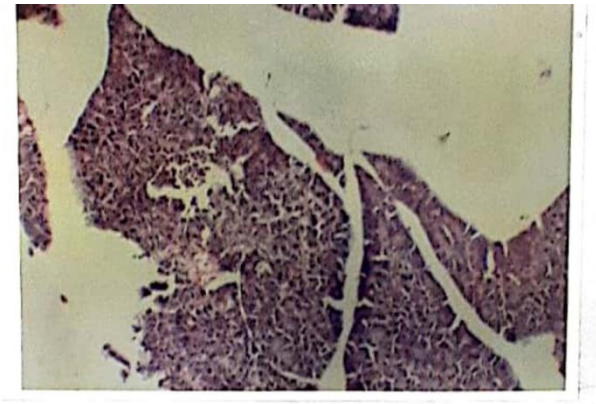


Table 2: Effect of *shilajatu* on Blood Glucose Level of allaxonised rats (Alloxan 150 mg/kg body wt.)
Route of Administration – Oral

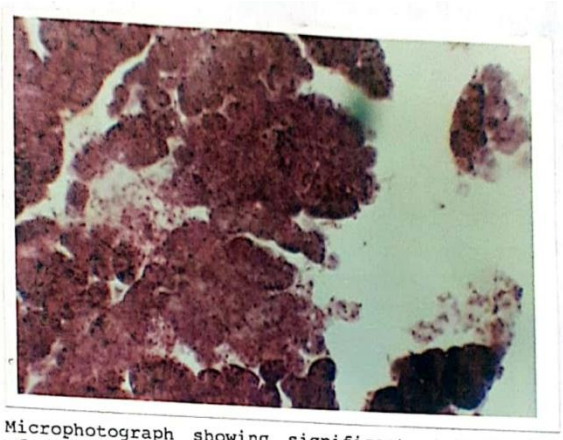
		AFTER SHILAJATU TREATMENT					
Groups	**Dose**	**Fasting blood sugar**	**48 hours**	**7 days**	**14 days**	**21 days**	**28 days**
Control	-	86.0±2.23 A	216.67±4.18 A1	222.83±6.78 A2	229.05±8.30 A3	222.11±7.27 A4	238.89±7.35 A5
l	200mg/kg	81.4±2.44 B	216.66±4.56 B1	208.26±8.33 B2	193.19±7.57** B3	174.99±6.80** B4	155.55±10.01** B5
ll	500mg/kg	82.62±2.61 C	209.93±4.86 C1	204.93±7.72 C2	190.53±4.92** C3	171.50±5.81** C4	146.63±2.72** C5
lll	1gm/kg	83.68±3.80 D	227.38±2.99* D1	200.38±5.43* D2	180.93±4.19** D3	170.83±6.84** D4	136.00±7.34** D5

* -- P‹ 0.005
** -- P‹ 0.0005

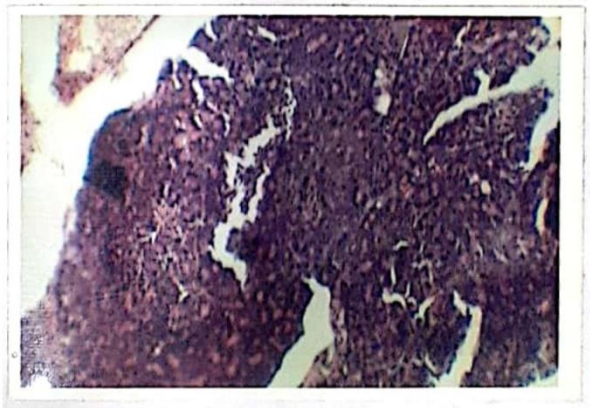




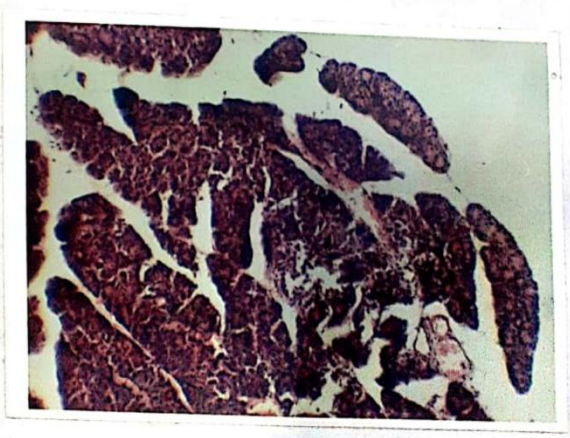
Microphotograph showing some amount of spontaneous cellular regeneration of Islet cell mass. Treated group II (200 mg/kg bd. wt.) H&E x 125.



Microphotograph showing significant regeneration of islet cell mass H&E stain x 125. Treated group IV (1000 mg/kg body wt.).



Microphotograph snowing some amount of cellular regeneration of Islet cell mass H&E x 125. Treated group III (500 mg/kg body wt.).



Microphotograph of pancreatic tissue showing significant regeneration of Islet cell mass in the treated group IV (1000 mg/kg body wt.) H&E x 125.

DISCUSSION

Commonly there is a trend in Ayurvedic society to use *shilajatu* shodhit by *triphala*[1] or *salsaradigana*[2] dravyas, but this study is planned to evaluate Madhumehagna (antidiabetic) property of *shilajatu*. *Triphala* or *salsaradi* have their own therapeutic property, hence to eliminate the bias in the experimental results, only water extracted *shilajatu* is selected for experimental study.

Though, we have collected three samples from the different places, but for experimentation we have selected only one sample procured from Nepal. This is because in our Pharmaceutical and spectral study sample of Nepal was found to be the best.

Histopathological study revealed that in alloxanised rats, massive necrosis of beta cells occurs in the islets of langerhans was observed after the 7th days which persists further upto the end of experiment. Hence it was clear from the above observation that alloxan causes selective beta-cells necrosis and produces permanent diabetes. Though, it is difficult to produce experimental diabetes exactly similar to the clinical diabetes which is a

syndrome that occurs spontaneously. However, alloxan induced diabetes is a standard experimental model.

In the second experiment i.e., effect of *shilajatu* on beta-cell regeneration, histopathological study in light microscope showed that, in control group out of five animals three of them showed necrosis of islet cells from 7th day to 28th day and no self regeneration was found. Rest of the two animals showed spontaneous regeneration. In treated groups (lower as well as higher doses) regeneration is more marked in comparison to control group. This might be due to the effect of *shilajatu*, because *shilajatu* has antioxidant property[3] which arrest aging process and induces rejuvenation.

In the *shialajatu* treated A.I.H. experiment the mean blood sugar level in control group showed rise from 7th day to 28th day. A slight decrease was observed on 14th day but it was statistically insignificant. Regular fall in the mean blood sugar level was observed in the entire treated group and data was insignificant in lower doses upto 2 weeks i.e. upto 14 days, but after 21st days it was found significant. In higher doses data was significant from the 7th day to 28th day. This significant hypoglycaemic effect of *shilajatu* is probably due to its antioxidant property. According to them *shilajatu* has significant effect against alloxan and streptozotocin induced hyperglycaemia by decreasing pancreatic islets superoxide dismutase, which is one of the supposed causative factor of damaging the beta-cells or this hypoglycaemic effect might be due to presence of zinc in shilajatu.[4] *Shilajatu* contains so many metals along with Zn in traces. Action of Zn in diabetes has been proved by many modern researchers.

According to them, Insulin is stored within the granules by the interaction of Zinc.

Lazarow (Ind.J.Med.Res.48:720, 1960) observed the prophylactic action of Zinc, if administered prior to alloxan.

Mirsky (1953) and Mirsky et.al. (1956) claimed that heavy metals such as copper, zinc etc. in vitro acted as insulinase inhibitors.

Proinsulin and insulin both have ability to bind zinc (Frank et. Al. 1968).

SUMMARY

The experimental study was performed in following ways:

1. To find out the effect of alloxan on the islets of langer hanse of beta cells within 28 days.
2. To find out the effect of shilajatu on the Alloxanised hyperglycemic rats.
3. To find out the effect of shilajatu on the damaged Beta Cells. The overall observations are summarized as follows

After injecting alloxan on healthy rats, they were hyperglycemic with in 48 hrs, and mean blood sugar level showed regular rise from 7th to 28th day. Histological study showed that there is a complete necrosis of islet cells and no further regeneration was seen till 28th days.

In the second experiment hyperglycaemia were produced in rats by alloxan and after 48 hrs shilajatu treatment was started. From this experiment we can conclude that in higher doses *shilajatu* showed significant hypoglycaemic action within 28th days.

In the third experiment it was obvious from the light microscopic examination that *shilajatu* showed less regenerative effect on beta cells but regeneration is not significant.

CONCLUSIONS

In higher doses, *shilajatu* shows significant hypoglycemic effect and may prevent maturity onset of diabetes by virtue of its Rasayan[5] effect.

In higher doses, *shilajatu* enhances the spontaneous regeneration of Beta-Cells. However, regenerative changes were minimal. This experiment was of preliminary in nature hence extensive studies are suggested.

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