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# EFFECT OF *STRYCHNOS POTATORUM LINN* ON REPRODUCTIVE FUNCTION IN FEMALE DIABETIC RATS

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**Keywords:**

Diabetes, Reproductive dysfunction, Streptozotocin, *Strychnos potatorum Linn*

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**ABSTRACT: Objective:** During the reproductive years, diabetes has been associated with menstrual abnormalities, sexual dysfunction and increases infertility rates among females. *Strychnos potatorum Linn (S. P. Linn*) has been recognized as antidiabetic drug as an Ayurveda medicine. This study was done to estimate whether *S. P. Linn* can control diabetes and prevent diabetes related complication in the reproductive system of female rats. **Method:** Experiments were carried out on 36 inbred female rats (150-200 gm) for studying the effect of the indigenous plant product on diabetic animal model. Six animals per cage were housed at room temperature with standard laboratory rodent pellet diet and water was allowed *ad libitum.* All animals were made diabetic by a single intraperitoneal Streptozotocin (STZ) injection at 40 mg/kg body weight, and then divided into six groups. Fasting blood sugar was estimated at biweekly interval. All the animals were sacrificed after three months. Uterus with fallopian tube and ovary were separated & weighed after sacrificing the animal on day 90. Ovary and vagina was collected for histopathological studies. **Result:** In the present study, *S. P. Linn* as an established insulinogenic hypoglycemic plant has reverted the reproductive dysfunction of the study animals. The results suggest that STZ-induced diabetic rats showed a significant reduction in weight of uterus with fallopian tube ($p< 0.01$) and ovary ($p< 0.01$). *S. P. Linn*, the test drug and Glipizide the standard hypoglycaemic drug were found to increase the uterus with fallopian tube and ovarian weight significantly ($p < 0.01$) in diabetic female rats. **Conclusions:** *S. P. Linn* has antidiabetic action on female diabetic rats. It significantly increased uterus with fallopian tube and ovarian weight in diabetic female rats which significantly get shrinked in diabetic state and probably reduced or revert the reproductive dysfunction of female diabetic rats.

**INTRODUCTION:** Diabetes mellitus is presently one of major global killer diseases.

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Worldwide, India is home to the second-largest number (77 million) of Diabetic adults [1]. India is expected to have 153 million diabetic population by 2045 [1]. WHO projects that diabetes death will double between 2005 and 2030 (WHO 2011) [2]. The International Diabetes Federation (IDF) estimated the total number of diabetic subjects in India to be around 40.9 million and importantly, further set to rise to 69.9 million by 2025 [3]. The disease in susceptible cases invites a series of complication

that may be acute or late in appearance, if not well controlled. Reproductive dysfunction is a well recognized consequence of diabetes mellitus both in male and female subjects. Accordingly, the therapeutic management of diabetes should include agents that are able to reduce the deleterious effects of diabetes on reproductive function. The goal of treatment of patients should be to achieve the best possible glycaemic control, restoring a normal, physiologic insulin response to food and decreasing the postprandial insulin levels and chronic hyperinsulinaemia [4]. Indigenous medicinal plants are undergoing extensive worldwide trials as because of their effectiveness, less expensive, less hazardous as compared to orally effective hypoglycemic drugs. Noteworthy effects of certain plants have been observed as antidotes of diabetes mellitus and infertility [5]. Indigenous medicinal plants are undergoing extensive worldwide trials as potential orally effective hypoglycemic agents capable of controlling diabetes mellitus *vis-a-vis* its complications and its distant influences [6, 7]. *Strychnos potatorum Linn.* has been used extensively as a folklore medicine and in Ayurveda practice for the treatment of diabetes mellitus especially in South India. An indigenous medicinal plant, *Strychnos potatorum Linn*. abundantly available in Central and Southern India [5] has been previously investigated in our laboratory and found to be hypoglycemic and insulinogenic [6, 7]. In experimental animal models of diabetes mellitus it has also been shown to reduce blood sugar level 8 and also reduce renal, hyperlipidemia, vascular and neurological complications in experimental animal models of diabetes mellitus.

The present study was undertaken to investigate whether *Strychnos potatorum Linn.* an indigenous hypoglycemic, insuligenic medicinal plant can control fasting blood glucose as well as reduce or prevent development of diabetes related complications in the reproductive system in diabetic female rats after obtaining approval from the ethics committee (MJNMCH / EC /17 / (2) dated 13.07.2022.).

## MATERIALS AND METHODS:

### Materials:

1. Animals
2. Drugs used
3. Chemicals and reagents
4. Preparation of drugs and reagents

**Animals:** Experiments were carried out on 36 inbred female rats (150-200 gm) for studying the effect of an indigenous plant product on the reproductive functions of a diabetic animal model. The animals were housed in cages of six animals per cage and a twelve-hour light and dark cycle at room temperature (25 ± 5°C) was maintained. They were fed on standard laboratory rodent pellet diet and water was allowed *ad libitum*.

**Drugs Used:**

- Streptozotocin (Sigma Chemicals)
- Glipizide- as tablets containing 5 mg per tablet (Franco India Ltd.)
- Indigenous-Drug *Strychnos potatorum Linn*, as dried seeds in powdered form.

**Chemicals and Reagents:**

- Citric acid and sodium citrate – for the preparation of acid citrate buffer
- Ether (Bengal Chemicals) for inducing anesthesia to the animals prior to sacrificing for the collection of viscera's and tissues for morphological examination.
- Distilled water (BDH chemicals)
- Sodium fluoride
- Gum acacia
- Hematoxylin
- Eosin
- Phosphate buffer saline (PBS).

**Preparation of Drugs and Reagents:**

**Acid Citrate Buffer:** To make a 50 mol of citrate buffer and the pH adjusted to 4.2, just before the experiment.

**Streptozotocin (STZ):** Streptozotocin (STZ) was kept at -20°C. It was dissolved in acid citrate buffer, adjusted to pH of 4.2 and immediately injected intra peritonially at a fixed dose schedule of 40 mg/ kg body weight in adult rats (0.1mL of acid citrate buffer contains 4mg of STZ).

**Indigenous Drug:** Dried nuts of *Strychnos potatorum Linn* (*S. P. Linn*) were crushed to form a fine powder. This fine powder was used for oral administration as 2% gum acacia suspension and fed orally at a dose of 100mg/kg body weight. The effective working dose was standardized in our laboratory by trial method.

**Glipizide:** The tablet was finely ground in a pestle and mortar and dissolved in 1.0 ml distilled water. The contents were then transferred to 1.0 ml syringe and fed with a rat cannula. Final washing with 0.5 ml of distilled water which was also fed orally to avoid wastage. The dose of 40 mg/kg body weight.

**Phosphate Buffered Saline (PBS), pH 7.4:**

**Methods:**

**Diabetic Study Group:** The study was undertaken in female diabetic rats. All animals were made diabetic by a single intraperitoneal injection of Streptozotocin (STZ) at a fixed dose of 40 mg/kg body weight. Animals were regularly checked. Mortality was approximately 25% and the blood glucose was estimated seven days after STZ injection. As we wanted to establish a Non Insulin diabetic animal model, animals with moderately altered blood glucose levels, ranging between 120 - 250 mg/dl were only studied whereas animals with blood glucose level above 250 mg/dl were not included in this study group.

Animals were then divided into 6 groups with 6 animals in each group.

**Group A:** Normal control animal.

**Group B:** Normal untreated animals who received the indigenous drug, *S. P. Linn*.

**Group C:** Normal untreated animals who received Glipizide, served as a standard drug control.

**Group D:** STZ induced diabetic animals served as Experimental control.

**Group E:** STZ induced diabetic rats, received the indigenous drug *S. P. Linn*.

**Group F:** STZ induced diabetic rats received Glipizide, a known hypoglycemic drug.

Body weight, fluid intake, and food intake were estimated weekly. Fasting blood sugar (FBS) estimated as biweekly interval. All the animals were sacrificed after three months. Blood was collected for final estimation of blood sugar. Ovaries, uterus with fallopian tube were collected from experimental rats for weight, morphology and histopathological examination.

**Detailed Methodology:**

**Fasting Blood Sugar:** Fasting blood sugar was estimated at the beginning, biweekly and at the end of the study.

**Method of Blood Collection:** Blood was collected from rat tails and from the retro–orbital venous plexus (Ghosh, 1984) [9].

**Estimation of Blood Glucose:** (Glucose Oxidase Method). *In-vitro* enzymatic colorimetric method for the quantative determination of glucose in serum/plasma (Span Diagnostics Ltd). The samples and reconstituted reagent were brought to room temperature prior to use. These were incubated at room temp for 30 minutes and optical density was measured at 510 nm against distilled water. The final color was stable for one hour.

**Weight of Ovary, Fallopian Tube and Uterus:** Ovary, fallopian tube and uterus were taken from lower abdomen after sacrificing the animal (on days 90). Organ weights were calculated per 100 gm of body weight.

**Histopathological Studies of Ovary:** The animal was anesthetized with open ether and sacrificed. Abdomen of the animals was opened by midline vertical incision. The ovary and uterus were identified and taken out for both macroscopic and microscopic examination (Chokroborty and Chokroborty, 1998) [10].

**Staining Method:** After collecting the organs, they were inspected macroscopically and weighed. They were fixed in 10% formalin over night and processed as follows. Sections from the organ after adequate fixation were dehydrated in graded alcohol, cleared in xylol and paraffin blocks were made. From this paraffin block, sections were made and fixed on the slides; the tissue was then stained accordingly (Chokroborty and Chokroborty, 1998) [10].

**Technique of Obtaining a Vaginal Smear:** A drop of water was placed in the vagina of the rat

with a medicine dropper. This was aspirated several times and then transferred to a slide where it was allowed to air dry. Then the smear was examined under the microscope and compared with stained samples (Ghosh, 1984) [9].

**Statistical Analysis:** The collected data were organized, tabulated, and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc., USA). Results were expressed as means ± SD. Statistical comparisons between groups were made by Student's t-test. P values of < 0.05 were considered statistically significant.

**RESULTS:** Effects of *S. P. Linn* and glipizide on fasting blood sugar of female rats in different treatment groups are depicted in **Table 1**. Significant changes observed in fasting blood glucose between day 0 and day 90 in all the groups except group A.

**TABLE 1: COMPARATIVE EFFECT OF *STRYCHNOS POTATORUM LINN* AND GLIPIZIDE ON THE FASTING BLOOD GLUCOSELEVEL IN DIFFERENT TREATMENT GROUPS (n =6)**

| Mean ± S.D of fasting blood sugar expressed in mg/dl | | |
|---|---|---|
| **Groups** | **Day 0** | **Day 90** |
| Normal control (Group A) | 80.83 ± 2.04 | 80.66 ± 3.72 |
| Normal control + *S. P. Linn* (Group B) | 79.83 ± 2.71 | 61.50 ± 6.74* |
| Normal control + Glipizide (Group C) | 80.66 ± 3.50 | 62.33 ± 5.22* |
| STZ-diabetic (Group D) | 180.33 ± 2.33 ** | 185.33 ± 4.50 ** |
| STZ + *S. P. Linn* (Group E) | 179.33 ± 2.73** | 78.00 ± 1.78$^{\dagger}$ |
| STZ+ Glipizide (Group-F) | 183.33 ± 6.88** | 72.66 ± 5.46$^{\dagger}$ |

**P < 0.01(as compared to normal); *P < 0.05 (as compared to normal); $^{\dagger}$P < 0.01(as compared to STZ treated); S.D = standard deviation, STZ = Streptozotocin.

Effects of *S. P. Linn* and glipizide on ovarian weight levels in different treatment groups as shown in **Table 2**. In STZ plus *S. P. Linn* group and in STZ plus Glipizide group the mean ovarian weight was significantly increased (p< 0.01) from day 0 to day 90.

**TABLE 2: EFFECTS OF *STRYCHNOS POTATORUM LINN* AND GLIPIZIDE ON OVARIAN WEIGHT LEVELS IN DIFFERENT TREATMENT GROUPS (n=6) AT DAY 90**

| Groups | Ovarian wt. in mg./100gm. body wt. (Mean ± S.D) |
|---|---|
| Group- A (Normal) | 40 ± 1.78 |
| Group-B (*S. P. Linn* treated) | 37 ± 2.68 |
| Group-C (Glipizide treated) | 36 ± 1.26 |
| Group-D (STZ treated) | 18 ± 0.89** |
| Group-E (STZ + *S. P. Linn*) | 35 ± 3.28$^{\dagger}$ |
| Group F (STZ + Glipizide) | 38 ± 3.57$^{\dagger}$ |

**p < 0.01(as compared to normal), $^{\dagger}$P < 0.01(as compared to STZ treated).

Effect of *S. P. Linn* and glipizide on weight of uterus with fallopian tube observed on Day 90 is depicted in **Table 3.** In STZ plus *S. P. Linn* group (Group D) and in STZ plus Glipizide group the mean uterus with fallopian tube mean weight was significantly higher than 205.3 ± 0.50 mg than STZ-diabetic group (p <0.01).

**TABLE 3: EFFECT OF *STRYCHNOS POTATORUM LINN* AND GLIPIZIDE ON UTERUS WITH FALLOPIAN TUBE WEIGHT IN DIFFERENT TREATMENT GROUPS (n=6) AT DAY 90**

| Groups | Uterus with fallopian tube wt. in mg/100gm. Body wt. (Mean ± S D) |
|---|---|
| Group- A (normal) | 242.1 ± 0.78 |
| Group- B (Normal + *S. P. Linn* ) | 241.2 ± 0.20 |
| Group- C (Glipizide treated) | 242.3 ± 0.54 |
| Group-D (STZ treated) | 205.3 ± 0.50** |
| Group-E (STZ + *S. P. Linn* ) | 245.6 ± 0.13$^{\dagger}$ |
| Group-F (STZ+ Glipizide) | 236.5 ± 0.18$^{\dagger}$ |

**p<0.01(as compared to normal), $^{\dagger}$P<0.01(as compared to STZ treated.

**Histopathological Observations of Ovary:** The section of the ovaries from the different groups shows similar features. Various stages of folliculogenesis from primary oocyte to graffian follicle were present. Atretic follicles, corpus luteum at varying stages of degeneration were identified. No obvious difference was noted within the groups.

**Histopathological Observations of Vaginal Smear:** All the four stages pro-estrus, estrus, metestrus, diestrus were seen in vaginal smear of all group of animal. No significant differences were observed in the different groups.

**DISCUSSION:** To study diabetes, experimentally induced diabetes is the preferred choice in animals as it is easy to establish diabetes within a short period of time (Rerup 1970 and Whitney *et al.*, 1982) [11, 12], have established a stable mild type of diabetes following i.p (intraperitoneal) administration of STZ which resembles NIDDM. Since this study focused on evaluating the therapeutic potency of the plant *S. P. Linn* in NIDDM, the streptozotocin induced diabetic model was considered as the most appropriate model of study in our laboratory. In this particular animal model, the diabetic state is maintained irrespective of the nutritional status of the animal (Kass *et al.*, 1945) [13] for a fairly long duration, moderate hyperglycemia is produced and associated and complications occur (Sarkar, 1999) [14].

In the present study, an effect of *S. P. Linn* was tried to establish as an insulinogenic hypoglycemic plant (Basu. 1992, Chatterjee, 1994) [15, 6]. *S. P. Linn* also known to arrest diabetic neuropathy (Sarkar, 1999) [14] and demonstrated profound hypolipidaemic activity (Chatterjee, 1997) [7]. Glipizide has been included as a standard drug for comparison because *S. P. Linn* acts by similar mechanism like glipizide i.e. inhibition of opening of ATP sensitive $K^+$ channels on beta cell membrane (Chatterjee, 1994) [6].

Controversies exist regarding change in body weight in STZ-induced diabetic rats. A gain in body weight was reported by Rakieten *et al.*, (1963) [16] and Kunjathoor *et al.*, (1996) [17] at a dose of 50 mg/kg b/w. STZ administered IV in rats whereas Lambert *et al.*, (1974) [18] reported weight loss after IV administration of 35 mg/kg b/w. in the same species. In this study, there was a marked decrease in body weight which is in agreement with the observation of previous workers (Biswas *et al.*, 1985, Goyal *et al.*, 1987) [19, 20]. In the current study, the significant and progressive loss of body weight was observed in Group D is possibly due to uncontrolled hyperglycemia which in turn leads to osmotic diuresis and glucose wastage (Foster, 1983) [21]. The observed gain in body weight in the *S. P. Linn* control group (Group B) can be attributed to either the resultant hypoglycemia (Basu, 1992, Chatterjee, 1994) [15, 6] which led to increased hunger and enhanced the food intake **Table 1**. A similar scenario has been reported in the DCCT trials (1993) [22] where the group of patients who received "high intensive therapy" ran a three-four higher risk of hypoglycemia which again was associated with a 1.6-fold higher risk of weight gain than in the conventional treated group.

Our results using *S. P. Linn* are in agreement with other studies undertaken in our laboratory (Basu, 1992, Chatterjee 1994, Chatterjee 1997**)** [15, 6]. Regarding the possible mechanism of the hypoglycemic activity of *S. P. Linn*., it has been suggested to have an insulinogenic action. The mechanism proposed is that like sulphonylureas, *S. P. Linn* causes an enhanced secretion of insulin by inhibiting the opening of the $K^+$ ATP sensitive channel (Basu 1992, Chowdhury 1995, Chatterjee, 1994) [15].

*Strychnos potatorum Linn* like glipizide demonstrated effectiveness in improving glycaemic control in diazoxide induced hyperglycemic rats. From the above study it can be summarized and suggested that the reduction of blood sugar level by *Strychnos potatorum Linn* is partly mediated through increased secretion of insulin by inhibiting opening of $K^+$ ATP channel caused by diazoxide (Chatterjee, 1994) [6].

**CONCLUSION:** *Strychnos potatorum Linn* has antidiabetic action on female diabetic rats. It significantly increased uterus with fallopian tube and ovarian weight in diabetic female rats which significantly get shrinked in diabetic state and probably reduced or revert the reproductive dysfunction of female diabetic rats.

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