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Antidiabetic Activity of *Spermadictyonsuaveolens*in Streptozotocin Induced Diabetic Rat Model.

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ABSTRACT

Aim of the study was to study effects of repeated dose administration of extract of roots of *Spermadictyonsuaveolens*in streptozotocin induced diabetic rats.Effects of extract of *Spermadictyonsuaveolens*wasstudied in streptozotocin-induced diabetic rats. 1 week after the administration of streptozotocin, diabetic rats received herbal extract orally at dose 500 mg/kg body weight for 28 consecutive days.Administration of streptozotocin resulted in 3-4 fold increase in fasting plasma glucose level. Treatment with herbal extract showed glucose lowering effect from first week. Results show that extract of roots of *Spermadictyonsuaveolens*possesses antidiabetic activity.

Keywords: Streptozotocin, *Spermadictyonsuaveolens,* Antidiabetic.

Introduction

Diabetes mellitus comprises a group of disorders that share the common feature of elevated blood glucose level.[1] The global burden of diabetes is high and rising in every country.[2] According to report of WHO/IDF (World Health Organization/International Diabetes Federation), in the year 2000 there were 171 million diabetic people in the world and this figure is estimated to increase to 366 million by 2030.[3]

Currently available therapies for the management of diabetes are associated with unwanted effects like hypoglycaemia, weight gain, acute pancreatitis, flatulence, nausea, vomiting, diarrhoea, etc. Currently available therapies are not proving sufficient to attain glycemic control in majority of diabetic patients.[4]

WHO suggested the evaluation of the potential of plants as effective therapeutic agents, for the chronic metabolic disorders like diabetes, hypertension etc, especially in areas where there is lack of safe modern drug.[5]

The plant *Spermadictyonsuaveolens*Roxb. [Synonym: *Hamiltoniasuaveolens* (Roxb.) Roxb.] belonging to family Rubiaceae is called as Jitasaya, Gidsawa, Gidesa, Raktarohada by local people.[6] The methanolic extract of leaves of *Spermadictyonsuaveolens*possesses wound healing activity.[7] The plant is used for the treatment of wound along with mustard oil. Grounded bark of the plant is rubbed on the body in puerperal fever. The root is used in the treatment of diarrhoea. The root infusion is given in courbature.[8]

In the present study, we studied acute oral toxicity and antidiabetic activity of extract of roots of *Spermadictyonsuaveolens.*

Material and methods

Animals:

Male and female Sprague Dawley rats weighing 200-250 g were procured from Bharat Serum and Vaccines, Thane. All animals were maintained in the SVKM's Animal Facility, Vile Parle (W), Mumbai, under standard conditions (temperature 25^0 C ± 2^0 C, relative humidity 75% ± 5% and 12 hour light/dark cycle). Rats were provided with standard laboratory feed and water ad libitum. Rats were allowed to acclimatize for one week before the onset of the experiment.[9] Protocols for toxicity study and efficacy study were approved by Institutional Animal Ethics Committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (Reference Number: CPCSEA/IAEC/BNCP/P-01 and CPCSEA/IAEC/BNCP/P-02)

Plant material:

The roots of *Spermadictyonsuaveolens*were collected from Dapoli, District: Ratnagiri, State: Maharashtra, India. The plant was authenticated at Blatter Herbarium, St. Xavier's College, Mumbai, India. The specimen matches with the Blatter Herbarium specimen number 11696 of H. Santapau. Roots were washed and dried in air at room temperature. Then roots were rubbed on stone with little distilled water. Paste so formed was dried in air at room temperature by spreading in petri plate. After evaporation of water, the extract obtained was triturated using mortar and pestle. As extract obtained was in powder form, it was suspended in distilled water using sodium carboxy methyl cellulose for oral administration.

Acute oral toxicity study:

The acute oral toxicity study was carried out using female Sprague Dawley rats according to OECD guideline 423 i.e. Acute Oral Toxicity – Acute Toxic Class Method. 9 female Sprague Dawley rats were randomly divided into 3 groups. The control group received distilled water and other two groups received the plant extract at the doses of 2000 mg/kg body weight and 5000 mg/kg body weight.[10] Animals were fasted overnight before administration of dose and were not fed for 3 hours following administration. Animals had free access to water. Animals were observed continuously for first 30 minutes and then periodically for first 24 hours with special attention during first 4 hours. After that, animals were observed daily for 14 days. Functional observation batteries such as convulsion, vomiting, diarrhoea, paralysis, breathing difficulties, bleeding, irritations and abnormal posture were observed. Body weights of animals were determined before administration of test substance and weekly thereafter. Animals were observed for signs of toxicity and mortality for 14 days with food and water intake.

Induction of diabetes in experimental animals:

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of STZ (Streptozotocin) in 0.1 M sodium citrate buffer (pH 4.5) at dose 55 mg/kg body weight. The same volume of buffer was administered to animals of control group. Animals were provided with 10% glucose solution after injection of streptozotocin. This decreased mortality in animals. The control group was provided with distilled water. After 3 days, plasma glucose levels were determined. Animals with plasma glucose levels more than 200 mg/dL were selected and used for further study.

Effect of repeated dose of extract of roots of *Spermadictyonsuaveolens*:

In this study we studied the effects of extract of *Spermadictyonsuaveolens*on streptozotocin-induced diabetic rats when administered orally for 4 weeks. Rats were kept for 1 week after injection of streptozotocin to stabilise the diabetic conditions and permanent hyperglycaemia. Then the treatment was begun. In the experiment, male Sprague Dawley rats were randomly divided in 4 groups with 6 animals in each group (n=6). Rats in normal control and diabetic control groups received sodium carboxy methyl cellulose in distilled water. One group of diabetic rats treated with extract of roots of *Spermadictyonsuaveolens* at dose 500 mg/kg body weight and one group of diabetic rats treated with metformin at dose 100 mg/kg body weight. (Metformin was obtained as a gift sample from Aarti Drugs Limited, Mumbai, India; Batch Number: MEF/15090169) Each rat of each group received vehicle or respective treatment for 28 consecutive days as a single daily dose orally.

Plasma glucose was checked weekly. For this, blood was collected in fasted animals. For blood collection, animals were given slight anaesthesia by diethyl ether inhalation. Blood samples were collected from retro-orbital plexus of rat. Blood was collected in eppendorf containing 10% EDTA (Ethylenediaminetetraacetic Acid) solution. Plasma was separated by centrifugation at 4000 rpm for 10 minutes. Body weight of all the experimental animals monitored weekly. Food intake, water intake, urine output and faecal matter output were recorded for all the groups. On day 28, animals were sacrificed by decapitation under anaesthesia. Estimation of glucose was done using commercially available diagnostic kit in ErbaChem 7 biochemistry analyser.

Histopathological studies:

On day 28, animals were sacrificed and rat pancreases were removed and stored in 10% neutral buffered formalin. Rat pancreases were given for histopathological evaluations at Chaitanya Laboratories, Pune. Pancreatic β cells were observed in histopathological studies.

Statistical Analysis: All data are expressed as mean ± SEM. The effect of treatment on body weight, food intake, water intake, urine output, faecal matter output were determined using one way analysis of variance (ANOVA) test followed by Tukey's honest test. Weekly glucose data was analysed using two way ANOVA test followed by Bonferroni test. P values less than 0.05 were considered significant.

Results

Acute oral toxicity:

The present investigation showed that the test extract at dose of 2000 mg/kg body weight and 5000 mg/kg body weight was non-toxic when administered as single oral dose. No mortality was recorded during the study. There was no change in food intake, water intake during the course of study. No behavioural modification was observed. Animals were sacrificed on the day 14 and macroscopic pathology was observed. There was no visible lesion in any animal. The oral LD_{50} (Lethal $Dose_{50}$) value of *Spermadictyonsuaveolens* root extract must be greater than 5000 mg/kg body weight.

Effect of repeated dose of extract of roots of *Spermadictyonsuaveolens*:

Body weight:

Table 1 shows the effect of repeated administration of extract of roots of *Spermadictyonsuaveolens* on body weight of control and treatment group rats. Induction of diabetes reduced body weight by 35-40 g. At the end of the study weight gain by diabetic control group was significantly lower as compared to non-diabetic groups. Treatment with extract of *Spermadictyonsuaveolens* showed improvement in body weight.

Table 1. Effect of treatment with extract of roots of *Spermadictyonsuaveolens* on weight gain in STZ-induced diabetic rats

Group	Weight gain (g)
Normal control	35 ± 3.022
Diabetic control	6 ± 1.265 a
Extract (500 mg/kg body weight)	25 ± 2.16 b
Metformin (100 mg/kg body weight)	16 ± 1.732

The values are expressed as Mean ± SEM at n=6

a: $p < 0.001$ compared to normal control; b: $p < 0.001$ compared to diabetic control

Effect on food intake, water intake, urine output, faecal matter output:

Table 2 shows the effect of repeated administration of extract of roots of *Spermadictyonsuaveolens* on food intake, water intake, urine output and faecal matter output of control and treatment groups rats. After induction of diabetes food intake, water intake, urine output and faecal matter output were significantly increased compared to non-diabetic rats. In both the treatment groups food intake, water intake, urine output and faecal matter output were significantly decreased as compared to diabetic control group.

Table 2. Effect of treatment with extract of roots of *Spermadictyonsuaveolens* on food intake, water intake, urine output and faecal matter output in STZ-induced diabetic rats

Group	Food intake (g/rat/day)	Water intake (mL/rat/day)	Urine output (mL/rat/day)	Faecal matter output (g/rat/day)
Normal control	16 ± 0.8165	20 ± 0.8944	14 ± 1.033	12 ± 0.9309
Diabetic control	34 ± 1 a	85 ± 2.129 a	65 ± 1.528 a	18 ± 1.095 a
Extract (500 mg/kg body weight)	18 ± 0.9309 b	24 ± 1.633 b	16.83 ± 1.447 b	11 ± 0.9661 b
Metformin (100 mg/kg body weight)	23 ± 0.8563 b	26 ± 1.612 b	23.5 ± 1.803 b	11 ± 1 b

The values are expressed as Mean ± SEM at n=6

a: $p < 0.001$ compared to normal control; b: $p < 0.001$ compared to diabetic control

Effect on plasma glucose level:

Table 3 shows the effect of repeated administration of extract of roots of *Spermadictyonsuaveolens* on plasma glucose level of control and treatment groups rats. Administration of STZ resulted in 3-4 fold increase in fasting plasma glucose level. There was significant increase in plasma glucose level ($p < 0.001$) in diabetic control as compared to non-diabetic group in all the weeks. Treatment with herbal extract showed glucose lowering effect from first week. Significant ($p < 0.001$) hypoglycaemic activity was exhibited by group receiving extract of

roots of *Spermadictyonsuaveolens* in week 1, week 2, week 3, week 4 as compared to diabetic control group. Treatment with metformin showed decrease in glucose level gradually. Metformin treated group showed significant ($p < 0.001$) anti-hyperglycaemic activity in week 2, week 3, week 4 as compared to diabetic control group. On day 28, reduction in plasma glucose was 57.1 % for group receiving extract of roots of *Spermadictyonsuaveolens*, 55.75 % for metformin treated group when compared with diabetic control group.

Table 3. Effect of treatment with extract of roots of *Spermadictyonsuaveolens* on fasting plasma glucose in STZ-induced diabetic rats (Plasma glucose level is expressed as mg/dL)

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Normal control	102.07 ± 3.05	107.07 ± 2.92	108 ± 2.68	103.02 ± 2.78	107.05 ± 3.14
Diabetic control	350.03 ± 7.48 a	320.05 ± 7.62 a	322.05 ± 7.63 a	335.01 ± 8.66 a	373 ± 10.9 a
Extract (500 mg/kg body weight)	338.58 ± 9.19	160.03 ± 7.88 b	158.03 ± 7.39 b	155.02 ± 7.19 b	160.03 ± 7 b
Metformin (100 mg/kg body weight)	335.02 ± 10.3	305 ± 8.38	255.06 ± 6.69 b	210 ± 6.29 b	165.05 ± 6.79 b

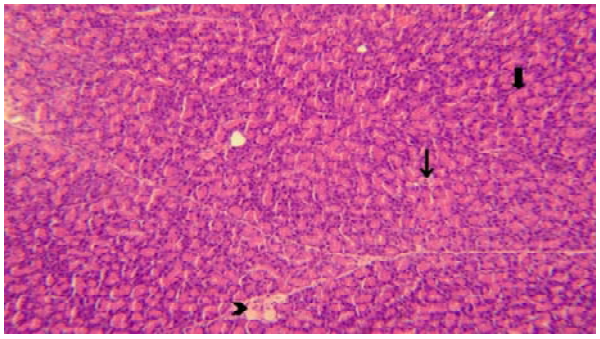
The values are expressed as Mean ± SEM at n=6

a: $p < 0.001$ compared to normal control; b: $p < 0.001$ compared to diabetic control

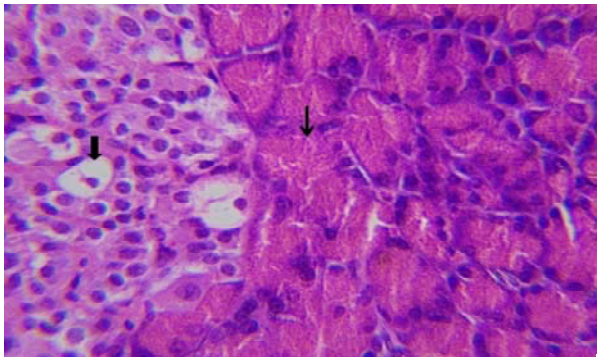
Histopathological studies:

Histopathology of all groups was studied (Figure 1). Non-diabetic group showed normal histology of pancreas. Diabetic group showed degenerative changes. In treatment groups restoration of degenerative changes were observed.

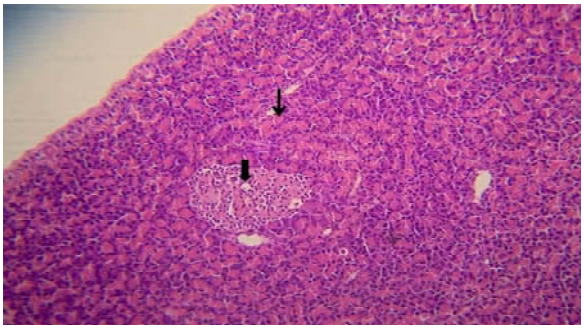
Figure 1 Histology



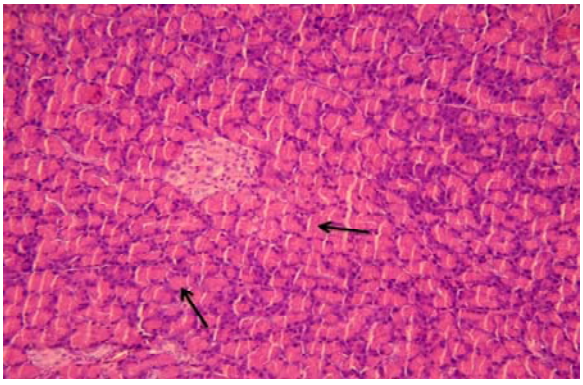
Normal control



Diabetic control



Extract (500 mg/kg body weight)



Metformin (100 mg/kg body weight)

Discussion

In this study, high urine output accompanied by higher intake of food and water indicated the diabetic state in animals due to administration of

streptozotocin. In STZ-induced diabetic rats, there is loss in body weight due to muscle destruction or degradation of structural proteins. Extract of roots of *Spermadictyonsuaveolens* is significantly effective in preventing polydipsia, polyphagia and polyuria. Despite the high feed and water intake in the untreated diabetic rats, the gain in body weight was very minimal compared to the treatment groups. The enhancement in body weight in herbal extract treatment group could be attributed to the increase in metabolic activity. This clearly indicates that the herbal extract increased glucose metabolism which enhanced body weight in rats.

In this study, high plasma glucose levels were observed due to administration of streptozotocin. Streptozotocin causes damage to pancreatic beta cells which leads to reduced levels of insulin and consequently results into hyperglycaemia.[11]The herbal extract could show its action by enhancing peripheral glucose uptake. In the 4 week treatment period, extract of roots of *Spermadictyonsuaveolens* displayed the significant decline in blood glucose level from first week. This indicates that the plant extract could contain hypoglycaemic compound. Metformin treated group showed gradual reduction in plasma glucose level over 4 weeks while plant extract showed immediate glucose lowering activity. So, the plant extract may improve hyperglycaemic conditions soon after the treatment has begun. The glucose lowering activity of extract of roots of *Spermadictyonsuaveolens*could be due to presence of flavonoids. Flavonoids act as antioxidant and contribute to anti-diabetic property.

Patho-morphological observation of pancreas, in present study suggests that administration of streptozotocin in diabetic control rats produced mild to moderate degenerative, necrotic and hyperplastic lesions. Treatment with extract of roots of *Spermadictyonsuaveolens* reduced the severity and distribution of pathological lesions incurred due to administration of streptozotocin.

Conclusion

The oral LD_{50} value of *Spermadictyonsuaveolens* root extract must be greater than 5000 mg/kg body weight. Results of the study shows that administration of streptozotocin induced hyperglycaemia, polyphagia, polydipsia, polyuria and loss in body weight. The oral administration of extract of roots of *Spermadictyonsuaveolens* significantly reduced plasma glucose level in streptozotocin-induced diabetic rats. Thus, extract of roots of *Spermadictyonsuaveolens*possesses antidiabetic activity.

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