Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 5 pp. 939–943, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

ANTIDIABETIC EFFECT OF SEEDS OF *STRYCHNOS POTATORUM* LINN. IN A STREPTOZOTOCIN-INDUCED MODEL OF DIABETES

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Abstract: The antidiabetic effect of seeds of *Strychnos potatorum* Linn. was evaluated in a model of diabetes mellitus using streptozotocin (40 mg/kg b.w., *i.p.*). Changes in fasting blood sugar were estimated periodically for 12 weeks along with weekly measurement of body weight, food and water intake for 4 weeks. The antidiabetic effects were compared with glipizide as the reference hypoglycemic drug. S*trychnos potatorum* Linn. (100 mg/kg *p.o.*) significantly reduced fasting blood sugar, the effects being comparable with glipizide (40 mg/kg, *p.o.*), an established hypoglycemic drug. It also increased body weight along with decreased food and water intake in streptozotocin-induced diabetic rats. Taken together, *Strychnos potatorum* Linn. shows promise as an effective hypoglycemic compound worthy of future pharmacological investigations.

Keywords: antidiabetic effect, fasting blood glucose level, streptozotocin, *Strychnos potatorum* Linn.

Diabetes mellitus is presently one of the major global killer diseases that affects approximately 5% of the total population (WHO, 1995) (1), with type 2 diabetes (NIDDM), accounting for more than 90 per cent of all cases. Nowhere is the diabetes epidemic more pronounced than in India, as the World Health Organization (WHO) reports indicate that 32 million people had diabetes in 2000 (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 complications that may be acute or late in appearance, precipitating morbidity that retards activities of normal life in relatively premature age groups and finally terminates in premature mortality, if not well controlled. Therefore, the goal of treatment should be to achieve the best possible glycemic control, restoring a normal, physiologic insulin response to food and decreasing the postprandial insulin levels and chronic hyperinsulinemia (4).

Indigenous medicinal plants are undergoing extensive worldwide trials as potential orally effective hypoglycemic agents capable of controlling diabetes mellitus vis-à-vis its complications and its distant influences (5, 6). The WHO expert committees (7, 8) have also suggested proper investigation and research amongst traditional methods of treatment for diabetes mellitus. In an ethnomedical field study of 98 medicinal preparations, involving 69 species of plants, used by Siddis of Uttara Kanada in the state of Karnataka, 40 hitherto unknown medicinal uses of known medicinal plants were identified (9).

Strychnos potatorum Linn. (Loganiaceae) is a moderate sized tree found in Central and Southern India as also in Sri Lanka and Myanmar; it has been used extensively as a folklore medicine and in ayurvedic practice, notably the fruit as an antidiabetic, antidystentric, emetic, while the pulp is useful as an expectorant (10). The seeds have been used as a demulcent, emetic, stomachic and tonic, and been found to be effective in the treatment of diabetes, diarrhea and gonorrhea; it has also been found to be locally effective for treatment of eye infections (10). Phytochemical studies revealed the presence of diaboline (major alkaloid) and its acetate, triterpenes and sterols and mannogalactans (11). The seeds have a wide spectrum of established pharmacological activity that includes anti-inflammatory (12),

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antihypercholesterolemic (13), diuretic (14), antidiarrhoeal (15), hepatoprotective (16), antinociceptive and antipyretic (17) as also antiulcer (18) activities. Furthermore, acute and chronic toxicity studies of aqueous extract and seed powder of *Strychnos potatorum* Linn. have established that the compound is non toxic up to 2.0 g/kg b.w. (19) and accordingly, the objectives of the present study was to validate its antidiabetic property in an experimental model of diabetes mellitus.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA) except glucose oxidase kit (Span Diagnostics, India).

Animals

Wistar rats (150–200 g) were housed in the institutional animal house maintaining a 12-h light and dark cycle and room temperature (25 ± 5°C); they were fed a standard pellet diet and water was provided *ad libitum*. The study was approved by the Institutional Animal Ethical Committee.

Preparation of *Strychnos potatorum* Linn. and glipizide

Dried nuts of *Strychnos potatorum* Linn. were procured from an authorized medicinal plant vendor, authenticated and a voucher specimen of the same was archived in the departmental museum. The dried seeds were crushed to form a fine powder, resuspended in 2% gum acacia suspension and fed orally (100 mg/kg b.w.). This effective working dose was standardized in our laboratory. Glipizide was dissolved in double distilled water and administered orally (40 mg/kg b.w.).

Induction of diabetes

Animals were rendered diabetic by a single *i.p.* injection of streptozotocin (STZ, 40 mg/kg b.w. in acid citrate buffer, pH 4.2). A severe hypoglycemia which generally occurs 5–8 h after administration of STZ was counteracted by giving 5% dextrose (20). Mortality was approximately 25% and the blood glucose was estimated seven days after STZ injection.

Study groups

Animals were divided into 4 groups of 10 animals each. Group A were non diabetic rats (normal control) while Groups B–D were rendered diabetic as described above. In the normoglycemic rats, Group A received the vehicle, 2% gum acacia suspension, while in the diabetic groups, Group B were the control STZ induced diabetic rats, while Group C animals received daily *Strychnos potatorum* Linn. (100 mg/kg b.w. in 2% gum acacia suspension, *p.o.*) and Group D rats received glipizide (40 mg/kg b.w.) for 12 weeks, respectively.

Estimation of fasting blood sugar

Overnight fasting blood samples were collected from the tail vein of all animals on day 0 (baseline) and at the end of week 4, 8 and 12 and levels of glucose were estimated by the glucose oxidase method as per manufacturer's instructions. In this assay, glucose oxidase is responsible for catalyzing the conversion of glucose into glucolactone along with conversion of oxygen into hydrogen peroxide.

Changes in body weight

Changes in body weight were recorded in overnight fasted rats that were weighed weekly during the initial period of the study i.e., on day 0, week 1, 2, 3 and 4; to measure the food intake, a measured amount of food was provided to the animals and at the end of 24 h, the remaining food was weighed and average consumption was calculated as:

$$\frac{\textit{Total food given} - \textit{Total food remaining}}{\textit{No. of animals in the cage}}$$

and expressed in g/animal/day; this was repeated once weekly for four weeks.

Statistical analysis

Results were expressed as the mean ± SEM; statistical comparisons between groups were made by paired *t* test and between groups by unpaired *t* test; $p < 0.05$ was considered statistically significant.

RESULTS

Effects of *Strychnos potatorum* Linn. and glipizide on fasting blood sugar

In Group A, the fasting blood sugar level showed minimal changes during the entire period of the study (Table 1). In the streptozotocin-treated group (Group B), the mean fasting blood sugar level at week 4 was significantly higher than for Group A (154.30 ± 1.41 mg/dL, $p < 0.01$, Table 1) and remained unchanged at week 12 (159.30 ± 0.75 mg/dL). In the STZ + *Strychnos potatorum* Linn. group (Group C), the mean fasting blood sugar levels at baseline (80.00 ± 3.2) significantly increased by week 4 (122.60 ± 1.78 mg/dL, $p < 0.01$), but was significantly lower than for animals which only

Table 1. Effects of *Strychnos potatorum* Linn. and glipizide on fasting blood sugar levels in an animal model of diabetes.

Group	Fasting blood sugar level (mg/dL) Mean ± SEM			
	Baseline	Week 4	Week 8	Week 12
A (Normal control)	70.00 ± 1.94	76.20 ± 1.45	79.30 ± 1.58	71.00 ± 1.5
B (STZ diabetic control)	74.00 ± 1.2	154.30 ± 1.41$^{\#}$	157.60 ± 1.54$^{\#}$	159.30 ± 0.75$^{\#}$
C (STZ + *Strychnos potatorum* Linn.)	80.00 ± 3.2	122.60 ± 1.78*$^{\#}$	113.60 ± 1.64*$^{\#}$	90.30 ± 1.92$^{\#}$ *
D (STZ + Glipizide)	78.00 ± 2.1	100.00 ± 1.43*$^{\#}$	108.00 ± 1.69*$^{\#}$	110.30 ± 1.5*$^{\#}$

Animals were made diabetic with streptozotocin (STZ, 40 mg/kg b.w., *i.p.*) and after oral administration of *Strychnos potatorum* Linn. (100 mg/kg) or glipizide (40 mg/kg), changes in fasting blood sugar were estimated on a monthly basis for 12 weeks as described in Materials and Methods. Results are expressed as the mean ± SEM of at least 6 animals per group.
$^{\#}p < 0.01$ compared to values at baseline of the same group; *$p < 0.01$ as compared to week-matched Group B.

Table 2. Effect of *Strychnos potatorum* Linn. and glipizide on body weight (g) in an animal model of diabetes.

Group	Baseline	Week 1	Week 2	Week 3	Week 4
A (Normal control)	110.0 ± 2.5	112.0 ± 5.2	115.0 ± 3.5	125.0 ± 4.8	130.0 ± 4.6
B (STZ diabetic control)	112.0 ± 4.8	111.0 ± 3.2	100 ± 2.7*	95.0 ± 4.7*	90 ± 2.9*
C (STZ + *Strychnos potatorum* Linn.)	108.0 ± 6.8	110 ± 3.9	118 ± 2.8†	125.0 ± 5.8†	130.0 ± 3.6†
D (STZ + Glipizide)	115.0 ± 4.7	118.0 ± 4.5	120.0 ± 2.4†	126.0 ± 3.6†	130 ± 2.9†

Animals were made diabetic with streptozotocin (STZ, 40 mg/kg b.w., *i.p.*) and after administration of *Strychnos potatorum* Linn. or glipizide, changes in body weight were estimated on a weekly basis for 4 weeks as described in Materials and Methods. Results are expressed as the mean ± SEM of at least 6 animals per group. *$p < 0.01$ compared to week-matched Group A; $^{\dagger}p < 0.01$ as compared to week-matched Group B.

received streptozotocin at the same time point (Table 1). Furthermore, the oral administration of *Strychnos potatorum* Linn. progressively decreased the fasting blood sugar values significantly at week 8 and 12 as compared to Group B at the same time points (Table 1). A similar trend was evident in Group D, as the addition of glipizide to STZ treated animals significantly decreased the fasting blood sugar level at week 4 as compared to Group B, at the same time point (100.00 ± 1.43 mg/dL *vs.* 154.30 ± 1.41, $p < 0.01$). Importantly, this trend was sustained at weeks 8 and 12 (Table 1).

Effect of *Strychnos potatorum* Linn. and glipizide on fasting body weight

The body weight of control rats (Group A), gradually increased with time and the animals appeared healthy and showed no external signs of disease (Table 2). However, in the streptozotocin treated rats (Group B), the weight progressively decreased, the decrease being significant at week 2, 3 and 4 as compared to Group A (Table 2); furthermore, the animals looked unhealthy with distinct loss of vitality and energy. In the STZ + *Strychnos potatorum* Linn. treated group (Group C), the initial body weight was 108.0 ± 6.8 g on day 0, which by week 4 increased to 130 ± 3.6 g; the decrease in body weight observed in Group B was prevented by administration of *Strychnos potatorum* Linn., as their body weight was significantly higher than Group B at weeks 2, 3 and 4 (Table 2); the rats were healthy and consumed their daily quota of food and water. A similar trend was observed in the STZ-diabetic group treated with glipizide (Group D, Table 2).

DISCUSSION

Diabetes mellitus, if not well controlled, invites a series of complications that precipitate morbidity leading to retardation of activities of normal life which eventually terminates in premature mortality. To study diabetes, experimentally induced diabetes is the preferred choice as it is easy to establish diabetes in animals within a short period of time and a stable mild type of diabetes following *i.p.* administration of STZ, which resembles non-insulin dependent diabetes mellitus (NIDDM), has been reported (21, 22). Since we were interested in evaluating the potential therapeutic effectiveness of the plant *Strychnos potatorum* Linn. in NIDDM, we considered this model as the most appropriate model of study.

The role of medicinal plants in the management of diabetes and its associated complications is currently a thrust area of medical research (5, 6). Acute and chronic toxicity studies of the aqueous extract and seed powder of *Strychnos potatorum* Linn. have indicated the absence of any toxicity up to 2.0 g/kg b.w. *p.o.* (19), furthermore, administration of *Strychnos potatorum*. Linn. (100 and 200 mg/kg b.w. *p.o.*) for 90 days to Wistar rats have been reported to have no significant effect on their food and water intake, body weight, hematological parameters, hepatic and renal functions, confirming its non toxic nature (19). Furthermore, its antidiabetic potential has been tested in alloxan-induced diabetic rats (23).

Controversies exist regarding change in body weight in STZ-induced diabetic rats. A gain in body weight has been reported (24, 25) at a dose of 50 mg/kg b.w. STZ administered *i.v.* in rats, whereas a weight loss after *i.v.* administration of 35 mg/kg b.w. has been reported in the same species (26). In our study, there was a marked decrease in body weight, which is in agreement with the observation of previous workers (27).

Initial phytochemical analysis of *Strychnos potatorum* Linn. revealed the presence of alkaloids (mainly diaboline) and four triterpenes from seeds and leaves (28). Subsequently, a detailed analysis was undertaken of the root, stem, bark and seeds, which provided evidence for the presence of alkaloids, flavonoids, glycosides, lignins, phenols, saponin, sterols and tannins (30). However, the phytoconstituents contributing towards the observed hypoglycemic effect are yet to be pinpointed. Previous studies have established the anti-arthritic, anti-inflammatory and antioxidant activity of *Strychnos potatorum* Linn. (31), which may be attributed to the presence of antioxidants such as flavonoids and phenols (30). Similarly, as the development of diabetes by streptozotocin is related to increased generation of free radicals (32), it may be extrapolated that the observed antidiabetic effect is mediated, at least partly, through its antioxidant effect. The ability of *Strychnos potatorum* Linn. to improve the glycemic response of STZ induced diabetic rats, along with its proven minimal toxicity indicates that it has promising antidiabetic activity meriting further pharmacological consideration.

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Received: 15. 06. 2011