Diabetes mellitus is one of the most common chronic diseases and a major contributor to vital organ damage and the development of cardiovascular diseases. It occurs due to deficiency or failure of normal action of insulin, which is responsible for use of sugar from the diet in the body. The number of cases of non-insulin dependent diabetes mellitus (type 2 diabetes) has increased dramatically due to changes in lifestyle, increasing prevalence of obesity (1). World Health Organization (WHO) predicts that close to four million deaths (6.8% of global all-cause mortality) in the 20–79 age groups in 2010 was due to this disease, which is 5.5% increase over the estimates for the year 2007 and predicted to double in 2030. The highest number of deaths due to diabetes is expected to occur in countries with large populations and low- and middle-income countries (almost 80% of diabetes deaths) and 12% increased deaths are reported in South East Asia from 2007 (International Diabetic Federation, report September 2009). In the absence of effective and affordable interventions for diabetes, the frequency of the disease will escalate worldwide, with a major impact on the population of developing countries (2–4).

In modern medicine, the beneficial effects of herbal medicines on glycemic level are well documented. The preventing activity of these drugs against progressive nature of diabetes and its complications was modest and not always effective. Insulin therapy affords effective glycemic control, yet its shortcomings such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage – fatal hypoglycemia limits its usage (5). Though sulfonylureas and biguanides are valuable in treatment of diabetes, their use is restricted by their limited action and side effects. For various reasons in recent years, the popularity of complementary medicines in diabetic control has increased (6). Natural plant drugs are frequently considered to be less toxic with lower side effects than synthetic ones (7). However, few have received scientific or medical scrutiny and the WHO has recommended that the traditional plant treatments for diabetes warrant further evaluation (8). This leads to increasing demand for herbal products with anti-diabetic activity and less side effects. In addition, the selection of herbal products in efficacy testing is relatively easy.

**ACUTE AND CHRONIC HYPOGLYCEMIC ACTIVITY OF SIDA TIAGHI FRUITS IN N5-STREPTOZOTOCIN DIABETIC RATS**

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**Abstract:** Herbal prescriptions have been recognized as potentially valid by the scientific medical establishment, and their use has been increasing. *Sida tiagii* Bhandari (*Sida pakistanica*; Family – Malvaceae), a native species of the Indian and Pakistan desert area, popularly known as “Kherenti” in India; is used as a folk medicine. In the present study, various fruit extracts of *Sida tiagii* were investigated for its hypoglycemic and antioxidant potential in neonatal streptozotocin-induced (type 2) diabetic rats. Grinded fruits were extracted with 90% ethanol and partitioned with n-hexane (n-Hexane Extract; HS) and ethyl acetate (Ethyl Acetate Extract; EAS) successively. The residual ethanol fraction (Residual Ethanol Extract; RES) was dried on water bath separately. All three extracts were administered orally at a dose of 200 mg/kg and 500 mg/kg. Blood glucose level, cholesterol, GSH (glutathione), elevated thiobarbituric acid-reactive substances (TBARS), glycated hemoglobin and liver glycogen contents were measured after 19 days treatment. The residual ethanol extract of *Sida tiagii* fruits significantly improve glycemic parameter and showed antioxidant activity in diabetic rats. The results of the present study indicated that the active fraction of *Sida tiagii* (i.e., RES) is suitable for development of a promising phytomedicine for the treatment of diabetes mellitus.

**Keywords:** type 2 diabetes, *Sida tiagii*, n-STZ rats

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because they have been used as folk medicines since long times (9, 10).

*Sida tiagii* Bhandari (*Sida pakistaniaca; Family – Malvaceae*), a native species of the Indian and Pakistan desert area, popularly known as “Kharenti” in India, is used in the folk medicine as/in blood purifier, tonic, muscle strengthener and treatment of metabolic disorders (11, 12). The species from *Sida* genus like *Sida cordifolia*, *Sida acuta*, *Sida rhomb-hoflia* and *Sida spinosa* are traditionally used as/in febrifuge, abortifacient, diuretic, metabolic disorders, dysentery, vomiting, gastric disorders, asthma, fever, aches, pains, ulcers, skin disease, diarrhea during pregnancy, rheumatism, neurological modulators and as anti-worm medication (13). These species have been known to possess antidiuretic, antidiabetic (14), anti-inflammatory, analgesic (15), antimalarial (16), antiplasmodial, hypotensive (17) and antibacterial properties (18). A recent report on *Sida tiagii* indicated that it contains terpenes, carbohydrates, flavones, resin and glycosides (19).

The literature review revealed that extracts of roots and leaves of these species of *Sida* were known to have various pharmacological activities (19–21), while fruits were screened only for its psychopharmacological activity, which is extensively used in traditional systems of medicine for treatment of metabolic disorders. In light of above, in the present study, we aimed to explore the beneficial effects of *Sida tiagii* fruits on n-STZ (neonatal streptozotocin)-induced diabetic rats by investigating their effects on carbohydrate metabolism, glycogen metabolism and antioxidant status.

**EXPERIMENTAL**

**Drugs and chemicals**

Tolbutamide, glibenclamide (Torrent Pharmaceutical, Ahmedabad), streptozotocin, heparin (SRL, India), EDTA (Hi-media Lab. Pvt Ltd., Mumbai), n-butanol, acetic acid, n-hexane, petroleum ether, ethyl acetate, glucose standard, citric acid, sodium citrate, Tris hydrochloride, buffer tablet, sodium lauryl sulfate, thiobarbituric acid, trichloroacetic acid, triton-X, glycerin, ethanol, Tween 80, carboxymethyl cellulose, Ellman’s reagent (5,5’-dithiobis-(2-nitro-benzoic acid) – DTNB), sodium sulfate, methanol, pyridine, anthrone, thiourea, benzoic acid, sodium chloride (s.d. Fine Chem Ltd., Mumbai).

**Preparation of extract of *Sida tiagii***

*Sida tiagii*, collected from the local fields of Rajasthan (India) in the month of October, was identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), India. *Sida tiagii* fruits were dried at 40 ± 1°C, ground into a granulated powder and defatted with petroleum ether. The ethanol extract was obtained by extracting 4 kg of defatted seed powder with ethanol (95%) at 50°C for 72 h in Soxhlet apparatus followed by filtration and concentration in rotary vacuum evaporator at 50 ± 5°C to its one third volume. The concentrate was partitioned with n-hexane (n-Hexane Extract; HS) and ethyl acetate (Ethyl Acetate Extract; EAS) and the respective layers were separated out and dried on water bath at 30°C till dryness (HS, 32.23 g, EAS, 26.68 g). The residual ethanol fraction (Residual Ethanol Extract; RES) was dried on water bath separately to yield RES (RES; 104.10 g). All the extracts were stored at the temperature below 10°C and were freshly prepared with 2% Tween 80 for pharmacological experiments.

**Experimental animals**

Wistar rats (2 day old pups with mother) were procured from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University, Hisar (Haryana). The mother rats were housed separately, one in a single cage (Polycarbonate cage size: 29×22×14 cm) under laboratory conditions with alternating light and dark cycle of 12 h each. The animals had free access to food and water. The animals were kept fasted 2 h before and 2 h after drug administration. The experimental protocol was approved by Institutional Animals Ethics Committee (IAEC) and animal care was taken according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436). The treatments were administered once a day orally.

**Induction of experimental neonatal streptozotocin diabetes**

Five-day-old male Wistar rats (pups) were fasted (separated from their mothers) for 8 h. One group of animals (diabetic, STZ) was injected with STZ (100 mg/kg, i.p.) freshly diluted in citrate buffer (10 mmol/L, sodium citrate, pH 4.5). The control group received only the vehicle solution in an equivalent volume. Mortality (44.6%) occurred in the STZ-treated group during the first day of injection. After weaning (day 21), the animals were kept in groups of 6 in collective cages under a 12-h light-dark cycle (lights on at 7:00 a.m.) at 23°C and with free access...
to food and water for the following 9 weeks and then used for study. Body weight and glycemia were weekly measured from weaning to sacrifice (12 weeks old). During the 10th week of age, the animals were kept in individual cages to determine blood glucose level and rats with the fasting plasma glucose level (PGL) of $\geq 200$ mg/dL were considered as diabetic and selected for further pharmacological studies (22).

**Acute toxicity study**

The rats were given all extracts (HS, EAS and RAS) at a dose 100, 200, 500, 1000 and 2000 mg/kg daily for a period of 3 weeks (five rats were taken for each group). The animals were observed for any physical sign of toxicity such as writhing, gasping, palpitation and decrease respiratory rate or motility.

**One day treatment study**

**Effect on blood glucose level:** Animals were divided in groups comprising of six animals per group as follows: (i) Non-diabetic (ND) control rats; (ii) Diabetic control: Animals were administered with vehicle only; (iii) Diabetic animals were administered tolbutamide (100 mg/kg; p.o.); (iv, v, and vi, vii) Diabetic animals were treated with 200 and 500 mg/kg, p.o – HS and EAS, respectively; (viii and ix) Diabetic animals were treated with 200 and 500 mg/kg p.o. RES, respectively. Blood glucose level was measured at 2, 4, 6 and 8 h after drug administration. Blood samples were taken from the tail vein (according to the Guideline 9 IACUC, 99). The glucose concentration was measured by Accu-check compact glucometers (Roche; GOD-POD Method).

**Effect on insulin secretion:** The extract RES showed optimum antihyperglycemic activity in initial screening at 500 mg/kg. Henceforth, HS and EAS were omitted for further study and RES (500 mg/kg) was used for further experiments. For acute effect on insulin secretion, animals were classified into five groups (1–5), each of which contained six rats. Group 1 (non-diabetic control) received vehicle, group 2 (non-diabetic) received the standard hypoglycemic agent, tolbutamide (100 mg/kg), group 3 (diabetic control) received vehicle, group 4 (diabetic) received tolbutamide (100 mg/kg), and group 5 (diabetic) received RES at a dose of 500 mg/kg. For the acute experiment, tolbutamide was selected as the control drug because Ohta et al. demonstrated in neonatal-induced streptozotocin diabetic rats that tolbutamide leads to increases in insulin secretion in early phase and this effect is not glucose-dependent (23). Serum insulin was measured by rat insulin ELISA kit at 0, 30, 60 and 120 min after administration of single dose (RES 500 mg/kg) in ELISA reader at 492 nm subtracting that at 630 nm (24, 25).

**Chronic experiment**

The diabetic animals were divided in two sets for oral glucose tolerance test and antihyperglycemic effect. Each set of animals was further divided in 4 groups comprising six animals in each, as follows: Group 1 (non-diabetic control) received vehicle, group 2 (diabetic control) also received vehicle, the rats of group 3 (diabetic) were given the standard oral hypoglycemic agent glibenclamide 600 µg/kg in the same vehicle, and group 4 (diabetic) received RES 500 mg/kg. All groups are subjected to 19 days treatment schedule.

**Oral glucose tolerance test:** After 19 days treatment, on 20th day, fasting rats were subjected to an oral glucose tolerance test (OGTT). Glucose (2 mg/kg) was administered to 18 h fasted rats. Blood samples were collected from tail vein at 0, 30, 60, 90 and 120 min.

**Chronic effect on blood glucose level and biochemical parameters:** Blood glucose level was measured on 5th, 10th, 15th and 20th day of treatment. The fasted animals were sacrificed by cervical decapitation on 20th day of the first treatment. Trunk blood was collected in heparinized tubes and the plasma obtained by centrifugation at 5000 rpm for 5 min was used for the determination of plasma cholesterol, malondialdehyde (MDA) and reduced glutathione, while whole blood was used for glycated hemoglobin. Total plasma cholesterol and glycated hemoglobin levels were measured by commercial supplied biological kit – Erba Kit (CHOD-PAP Method) using Chem 5 Plus-V2 auto-analyzer (Erba Mannheim Germany) in plasma sample prepared as above. Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Okhawa et al. (26). Estimation of plasma reduced glutathione level was performed as previously reported (27). Liver glycogen estimation was done by the method as described (28).

**Data analysis**

All the results were expressed as the mean ± standard error of the mean (SEM). The data of all the groups were analyzed using one-way ANOVA followed by Dunnett’s t-test using the software Sigma-Stat 3.5. In all the tests, the criterion for statistical significance was p < 0.05.
RESULTS

Toxicity study

The rats tested with all doses of HS, EAS and RES did not show any drug-induced physical sign of toxicity during the whole experimental period and no deaths were registered.

| Table 1. Effect of *Sida tiagii* on STZ diabetic rats on acute hyperglycemic model. |
|------------------------------------------|-------------------------------------|-----------------|-----------------|-----------------|
| Treatment                              | % reduction plasma glucose level     | 2 h             | 4 h             | 6 h             | 8 h             |
| Diabetic control                        |                                     | 0.63 ± 6.94     | 0.34 ± 5.21     | −1.00 ± 14.28   | 1.60 ± 4.84     |
| HS 200 mg/kg                            |                                     | −5.29 ± 5.34    | −7.24 ± 3.14    | −6.98 ± 3.78    | −7.04 ± 4.01    |
| HS 500 mg/kg                            |                                     | −6.52 ± 7.16    | −8.12 ± 2.15    | −9.74 ± 4.91    | −7.63 ± 6.87    |
| EAS 200 mg/kg                           |                                     | −9.11 ± 6.24    | −11.74 ± 2.37   | −14.57 ± 7.76   | −18.14 ± 5.34   |
| EAS 500 mg/kg                           |                                     | −11.64 ± 2.56   | −12.25 ± 9.34   | −31.27 ± 4.34** | −27.79 ± 3.65   |
| RES 200 mg/kg                           |                                     | −19.17 ± 8.61*  | −23.95 ± 4.27** | −37.46 ± 3.96** | −39.31 ± 7.42** |
| RES 500 mg/kg                           |                                     | −27.21 ± 9.04*  | −34.77 ± 6.34** | −43.87 ± 7.64** | −42.62 ± 4.87** |
| Tolbutamide                             |                                     | −31.51 ± 5.79** | −47.53 ± 3.29** | −57.76 ± 6.34** | −56.69 ± 9.58** |

Values are presented as the mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett’s test; * p < 0.05; ** p < 0.01; HS: n-hexane fraction of extract; EAS: ethyl acetate extract; RES: residual ethanol extract; tolbutamide (100 mg/kg).

| Table 2. Effect of *Sida tiagii* on acute insulin secretion. |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Treatment                        | Plasma insulin level (ng/mL) |
|                                 | 0 min                  | 30 min                | 60 min                  | 120 min                  |
| Control (−) ND                 | 2.015 ± 0.22            | 1.780 ± 0.20          | 2.200 ± 0.48            | 1.920 ± 0.22              |
| Control (−) ND Tolbutamide    | 2.408 ± 0.52            | 3.010 ± 1.22**        | 5.223 ± 0.82***         | 4.230 ± 0.52***           |
| Control (+) D                  | 2.118 ± 0.10            | 2.089 ± 0.10          | 2.361 ± 0.38            | 2.118 ± 0.10              |
| (+) D Tolbutamide             | 2.540 ± 0.42            | 4.210 ± 0.92**        | 5.036 ± 0.90***         | 3.960 ± 0.48**            |
|                                 | 2.010 ± 0.18            | 3.702 ± 0.42**        | 3.198 ± 0.24**          | 2.010 ± 0.18              |

Values are presented as the mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett’s test; a p < 0.05; b p < 0.01 when compared to control (−) D control with respective time; * p < 0.05; ** p < 0.01 when compared to 0 h with respective group; ND: non-diabetic; D: diabetic; RES: residual ethanol extract; tolbutamide (100 mg/kg).

| Table 3. Effect of RES on glucose level after 19 day treatments. |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Treatment                        | Plasma glucose (mg/dL) level on |
|                                 | 0 day                  | 5th day               | 10th day                | 15th day                | 20th day                |
| Diabetic control                | 210 ± 4.28             | 211.323 ± 7.34        | 212.667 ± 5.01          | 207.9 ± 10.27           | 215.838 ± 6.79          |
| RES 500 mg/kg                   | 206.270 ± 3.90         | 167.272 ± 3.96**      | 147.351 ± 3.16**        | 124.032 ± 4.28**        | 108.850 ± 3.41**        |
| Glibenclamide                   | 212.014 ± 6.37         | 167.034 ± 2.29**      | 135.362 ± 6.37**        | 120.055 ± 5.23**        | 87.852 ± 6.02**         |

Values are presented as the mean ± SEM; n = 6 in each group. One way ANOVA followed by Dunnett’s test; ** p < 0.01; RES: residual ethanol extract; glibenclamide (600 µg/kg).

RESULTS

Toxicity study

The rats tested with all doses of HS, EAS and RES did not show any drug-induced physical sign of toxicity during the whole experimental period and no deaths were registered.

Effects of one day treatment

Administration of various fruit extracts of *Sida tiagii* with a dose of 200 mg/kg and 500 mg/kg to n-STZ diabetic rats showed a variable effect on glucose level. HS (200 and 500 mg/kg) and EAS (200 mg/kg) treatment does not showed glucose reducing activity in acute hyperglycemic study, whereas EAS
Acute and chronic hypoglycemic activity of *Sida tiagii* fruits...

Figure 1. Values are presented as the mean ± SEM; n = 6 in each group. PG: plasma glucose, RES: residual ethanol extract, Gli: glibenclamide

Figure 2. Effect of RES on glycated hemoglobin level. Values are presented as the mean ± SEM; one way ANOVA followed by Dunnett’s test; a: p < 0.05, b: p < 0.01 when compared to normal rats values, ** p < 0.01 when compared to diabetic control group. Nor: Normal rat; Dia Cont: Diabetic control; Gli: Glibenclamide (600 µg/kg), RES 500: Residual ethanol extract (500 mg/kg)

Figure 3. Effect of RES on total plasma cholesterol level. Values are presented as the mean ± SEM; one way ANOVA followed by Dunnett’s test; a: p < 0.05 when compared to normal rats values; ** p < 0.01 when compared to diabetic control group. Nor: Normal rat; Dia Cont: Diabetic control; Gli: Glibenclamide (600 µg/kg), RES 500: Residual ethanol extract (500 mg/kg)

Figure 4. Effect of RES on liver glycogen content level. Values are presented as the mean ± SEM; one way ANOVA followed by Dunnett’s test; a: p < 0.05, b: p < 0.01 when compared to normal rats values; * p < 0.05, ** p < 0.01 when compared to diabetic control group. Nor: Normal rat; Dia Cont: Diabetic control; Gli: Glibenclamide (600 µg/kg), RES 500: Residual ethanol extract (500 mg/kg)

Figure 5. A. Effect of RES on plasma malondialdehyde level. B. Effect of RES on plasma GSH level. Values are presented as the mean ± SEM; one way ANOVA followed by Dunnett’s test; a: p < 0.05, b: p < 0.01 when compared to normal rats values; ** p < 0.01 when compared to diabetic control group. Nor: Normal rat; Dia Cont: Diabetic control; Gli: Glibenclamide (600 µg/kg), RES 500: Residual ethanol extract (500 mg/kg); GSH: plasma glutathione
(200 mg/kg) exhibited significant hypoglycemic activity after 6 h of administration. It seems that the onset of action was near to 6 h. RES significantly reduced the blood glucose level at both 200 and 500 mg/kg doses. The hypoglycemic activity of RES was time dependent and found maximum between 6 and 8 h after its administration. The onset of action was observed near to 2 h in the case of RES. RES showed similar results as tolbutamide (Tab. 1).

The insulin level of non-diabetic rats group increased compared of control after 30 min administration of tolbutamide (100 mg/kg) and this was observed up to 120 min. The insulin level of diabetic rats was also found to be increased after 30 min of tolbutamide administration and was significant till 120 min. Similarly, the plasma insulin was found to increase significantly compared to diabetic control after oral administration of RES (500 mg/kg) (Tab. 2).

Effects of chronic treatment

Effect on blood glucose: HS and EAS did not show significant glucose lowering potential in acute experimentation, henceforth were omitted from further chronic experimentation. RES (500 mg/kg) showed optimum result in one day treatment and was further included in chronic study. RES showed significant reduction (p < 0.01) in blood glucose level at 500 mg/kg, p.o. dose at all days (Tab. 3).

Oral glucose tolerance test: RES improved glucose tolerance at both doses, comparable to glibenclamide. The glucose clearance rate was faster in diabetic rats treated with RES than the diabetic control rats (Fig. 1).

Effects of RES on glycated hemoglobin, total cholesterol and liver glycogen content: Figure 2 depicted the effects of RES (500 mg/kg) on glycated hemoglobin (HbA1c). The HbA1c data for the n-STZ diabetic group were significantly higher than for the control (non-diabetic group). RES showed significant reduction of glycated hemoglobin level. The chronic administration of the reference drug, glibenclamide, significantly lowered the HbA1c in 20 days. The data depicted in Figure 3 indicated the effect of RES on total plasma cholesterol. It was significantly higher (113.74 ± 6.77 mg/dL; p < 0.01) in diabetic rats compared to normal rats (65.80 ± 3.30 mg/dL). The chronic administration of the reference drugs significantly (p < 0.01) lowered the total plasma cholesterol concentration. The administration of RES at 500 mg/kg showed significant reduction in total plasma cholesterol to 81.24 ± 3.29 mg/dL level from 113.74 ± 6.77 mg/dL. The liver glycogen level was found to be low in n-STZ rats compared to normal rats (Fig. 4). RES, at a dose of 500 mg/kg, increased the liver glycogen content significantly compared to diabetic control rats (p < 0.05).

Effects of RES on antioxidant parameters: The data indicated in Figure 5a demonstrate the plasma level of MDA in normal and experimental animals in each group. Plasma MDA level was found to be significantly higher in n-STZ diabetic rats compared to normal rats. Treatment of n-STZ diabetic rats with RES resulted in marked decrease in plasma MDA level, which was comparable to that of standard glibenclamide. The plasma glutathione (GSH) level was significantly decreased in n-STZ rats compared to control animals. The level of glutathione was returned to near normal range in n-STZ diabetic rats treated with RES and diabetic rats treated with glibenclamide (Fig. 5b).

DISCUSSION

The present study was conducted to access the antihyperglycemic activity of Sida tiagii fruit extract on n-STZ diabetic rats. n-STZ diabetic model is widely employed for screening of type 2 antidiabetic activity. As previously discussed, tolbutamide leads to increased insulin secretion in n-STZ diabetic rats independent of blood glucose concentration and has been used in acute study as reference antidiabetic drug. From Table 1, it can be concluded that n-hexane extract of Sida tiagii (HS) has no hypoglycemic activity. Ethyl acetate extract (EAS) showed little effect (31.27 ± 4.34% reductions in PG) at higher dose (500 mg/kg) at 6 h; it indicated that EAS may have low concentration of active principle. RES at doses of 200 and 500 mg/kg showed significant reduction of elevated glucose level on single administration. The onset of action of residual ethanol extract of Sida tiagii (RES) starts at 2nd h and persisted till 8 h. The peek glucose reduction (43.87 ± 7.64% reduction in plasma glucose) was observed at 6 h on treatment with RES (500 mg/kg). The acute effect of RES is further supported by its insulin secretagogues activity.

Further, during chronic study in RES treated rats, at doses of 500 mg/kg, significant reduction in plasma glucose level was observed on 19 day of administration with 47.16 ± 3.41% reductions in PG. In the RES treated diabetic rats, improved glucose tolerance was observed as reported by Ravikant and Diwan on using Sida cordifolia (Family – Malvaceae) (15). Furthermore, here we confirmed the previous observation in uncontrolled and long term diabetes that an increased glycosylation of a number of pro-
teins, including hemoglobin, were observed along with higher levels of glucose (29, 30). HbA1c makes up 3.4–5.8% of total Hb in normal human red blood cells, but it is increased in patients with overt diabetes mellitus (31). Glycated hemoglobin was found to increase in diabetic patients up to 16% and the level of HbA1c is monitored as a reliable index of glycemic control in diabetes (32). In case of RES treated rats (500 mg/kg), glycated hemoglobin was reduced to 8.06 ± 0.84% of Hb level from that of 12.36 ± 0.91%. The above result indicated that RES has good glycemic control in long term diabetics. The cholesterol reducing activity of RES may be explained on the basis of improved glycemic control. Similar beneficial results were observed with live glycogen, which may be attributed to improved glycemic control or effect on gluconeogenesis, which needs to be further investigated.

Auddy reported antioxidant potential in Sida genus and similar results were observed in the present study (33). RES (500 mg/kg) showed strong antioxidant potential with reduction of MDA level. Increasing evidence in both experimental and clinical studies suggested that oxidative stress plays a major role in the development and progression of both type 1 and type 2 diabetes mellitus. The facts that the role of antioxidant compound in both protection and therapy of diabetes mellitus were also emphasized by previous scientific reports. There is an evidence that glycosylation of various proteins may itself induce the generation of oxygen-derived free radicals under diabetic condition. Hyperglycemia results in the generation of free radicals, which can exhaust antioxidant defense thus leading to the disruption of cellular functions, oxidative damage to membrane and enhance susceptibility to lipid peroxidation. This can be supported by present results, where significant increase of plasma MDA concentration was observed in case of diabetic rats. It has been reported that the products of lipid peroxidation diffuse from the site of tissue damage and therefore can be measured in plasma (34–36).

In the present study, decreased plasma glutathione level was observed in n-STZ diabetic rats (13.39 ± 3.22 mg/dL) compared to normal rats (38.54 ± 2.83 mg/dL). Reduced glutathione has an important role in regulation of cellular redox state and therefore imbalance in reduced glutathione to oxidized glutathione is a putative indicator of cellular oxidative stress (37). A decrease in plasma glutathione level of n-STZ diabetic rats is partly due to its utilization by the tissue to compromise the deleterious effect of lipid peroxidation (38). There is a negative correlation between GSH and HBA1c in diabetic animals as reported by Giugalino, which conforms the link between hyperglycemia and GSH depletion (34, 39) and is supported by our observation. RES was observed to restore depleted GSH at 500 mg/kg doses, which was comparable to the standard drugs glibenclamide. Restored GSH and decreased glycated hemoglobin on treatment of RES confirms the aforementioned hypothesis. Indeed, under hyperglycemic conditions, glucose is preferentially utilized in polyol pathway, which consumes NADPH necessary for GSH regeneration by the GSH-Red enzyme (39). Hyperglycemia is therefore indirectly the cause of GSH depletion. The RES caused a decrease of glycated hemoglobin and increased hepatic glycogen less than the reference drug, glibenclamide, but at the same time decreased MDA content more than the reference drug, which may be attributed to its direct antioxidant activity and independent to glucose level.

The phytoconstituents responsible for the anti-hyperglycemic and hypolipidemic effects of Sida tiagii include polysaccharides, flavonoids, alkaloids, diterpenes, resin, amino acid derivatives and steroidal glycosides (40, 41). The detailed phytochemical investigation has not been reported yet. On the basis of preliminary phytochemical investigation of Sida tiagii, more polar compounds such as alkaloids and flavonoids are responsible for an increase in insulin secretion and peripheral glucose utilization (19).

In conclusion, the residual ethanol extract of Sida tiagii fruits has potential hypoglycemic activity in n-STZ diabetic rats. The mechanism of action of beneficial effect action may be attributed to involvement of RES in improved insulin secretion and peripheral glucose utilization. Further, phytochemical and pharmacological evaluations have to be carried out on the Sida tiagii in order to identify the active principle responsible for antihyperglycemic activity and its mechanism of action.

Acknowledgment

This study was supported by Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India.

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Received: 6. 05. 2011