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Antidiabetic and Antioxidant Activity of *Dregea volubilis* Fruit in Streptozotocin-Induced Diabetic Rats

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Present study evaluated antidiabetic and antioxidant effect of petroleum ether extract of *Dregea volubilis* fruit in Wistar rats. Hyperglycemia was induced in rats by single intraperitonial injection of streptozotocin (STZ, 65 mg/kg body weight). After 3 days, streptozotocin induction, the hyperglycemic rats were treated with petroleum ether extract of Dregea volubilis orally at 100 and 200 mg/kg body weight daily for 15 days. Glibenclamide (0.5 mg/kg, orally) was used as reference drug. The fasting blood glucose levels were measured on every 5th day during the 15 days treatment. Serum and liver biochemical parameters were estimated. petroleum ether extract of Dregea volubilis significantly ($p < 0.001$) and dose dependently normalized blood glucose levels as compared to that of streptozotocin control. Serum and liver biochemical parameters were significantly ($p < 0.001$) restored towards normal in petroleum ether extract of Dregea volubilis treated rats as compared to streptozotocin control. Present study infers that *Dregea volubilis* fruit demonstrated remarkable antidiabetic and antioxidant activity in streptozotocin -induced diabetic rats.

Key Words: Antidiabetic, Streptozotocin, Glibenclamide, Lipid peroxidation.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosurea and negative nitrogen balance and it is mainly due to absolute deficiency or diminished effectiveness of insulin. It is the most prevalent disease in the world affecting 25 % of population and afflicts 150 million people and would rise to 300 million by 2025[1]. It causes number of serious complications like retinopathy, neuropathy and peripheral vascular insufficiencies[2].

Diabetes is still not completely curable by the present antidiabetic therapy. Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment[3,4]. There are several oral hypoglycemic agents used therapeutically but certain adverse effects and weak effectiveness of them led to search for more effective agents. Investigation in the plant kingdom culminated in the discovery of many natural antihyperglycemics[5].

Dregea volubilis Benth (Asclepiadaceae), commonly known as Jukti in Bengali, is a tall woody climber with densely lenticulate branches, occurring throughout the hotter parts of India and Car Nicober Islands ascending to an altitude of 1500 m. The parts of the plant have been traditionally used for medicinal purposes. The juice of the plant is used as a sternutatory and leaves are employed in application for boils and abscesses. The roots and tender stalks are used as emetic and expectorant. It is reported that an alcoholic (50 %) extract of the plant showed activity on the central nervous system as well as anticancer activity against Sarcoma 180 in mice. Two pregnane glycosides dregeosides were isolated from this plant collected from Thailand showed antitumor activities against melanoma B-16 in mice[6]. The isolation and characterization of twelve polyhydroxy C/D *cis*-pregnane glycosides were reported from the same plant collected from Thailand[7]. Isolation of β-sitosterol, kaempherol-3-galactoside, a 2-deoxy sugar, drevogenin A, drevogenin P, D-cymarose and L-olendrose from the plant was also reported[8]. The authors reported the isolation and characterization of a novel pentacyclic triterpenoid designated as taraxerone having antileishmanial and anticancer activity on K562 leukemic cell line[9]. No pharmacological investigation is still reported on *Dregea volubilis* fruits. Present

study was therefore aimed to investigate the antidiabetic effect of petroleum ether extract of *Dregea volubilis* fruit (PEDV) against streptozotocin (STZ)-induced diabetic Wistar rats.

EXPERIMENTAL

The fruits of *Dregea volubilis* were collected during August 2008 from South 24-Paraganas, West Bengal, India. The plant material was taxonomically identified by Dr. Lakhmi Narashimhan, Scientist, Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/I-I/(267)/2008/Tech.II/267] was maintained in our laboratory for future reference. The fruits were shade-dried with occasional shifting and then powdered with mechanical grinder passing through sieve No. 40 and stored in an air-tight container.

Streptozotocin (STZ), 5,5-dithio *bis*-2-nitro benzoic acid (DTNB), reduced glutathione (GSH), nitroblue tetrazolium (NBT): SISCO Research Laboratory, Mumbai, India; thiobarbituric acids, trichloroacetic acid (TCA): Merck, Mumbai; potassium dichromate, glacial acetic acid: Ranbaxy, Mumbai; glibenclamide; Hoechst, India. All other reagents used were of analytical grade obtained commercially.

Preparation of extract: The powdered plant material (450 g) was extracted with petroleum ether (60-80°C) for 72 h in the cone shaped percolator at 33 °C. The solvent was distilled in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue (petroleum ether extract of *Dregea volubilis*, 5.33 % w/w). Preliminary phytochemical studies performed on petroleum ether extract of *Dregea volubilis* revealed the presence of alkaloids, triterpenoids and steroids[10].

Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2 ºC with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to experiment. All procedures described were reviewed and approved by the University Animal Ethics Committee, Jadavpur University.

Acute toxicity: The acute oral toxicity of petroleum ether extract of *Dregea volubilis* in male Swiss albino mice was studied as per reported method[11].

Induction of diabetes: Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin (65 mg/kg body weight)[12]. After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level ≥ 225 mg/dL were used for the present investigation[13].

Treatment schedule and estimation of fasting blood glucose (FBG) level: The rats were divided into five groups (n = 6). Except group I which served as normal non-diabetic control all other groups were comprised of diabetic rats. Group II served as diabetic (streptozotocin) control. Group III and IV received petroleum ether extract of *Dregea volubilis* (100 and 200 mg/kg b.w., p.o., respectively) and group V received reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. Fasting blood glucose was measured on 0, 5th, 10th and 15th day by using a one touch glucometer (Accu-check®). 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum biochemical parameters *viz.*, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (SALP), serum cholesterol and total protein. Then the rats were sacrificed by cervical dislocation for the study of liver biochemical parameters like lipid peroxidation, reduced glutathione and catalase[14].

Body weight, liver and kidney weights: The body weight of rats of each group were measured just before and 15 days after petroleum ether extract of *Dregea volubilis* treatment. Liver and both kidney weights of all rats were measured after post treatment sacrifice.

Estimation of serum biochemical parameters: Collected blood was used for the estimation of serum biochemical parameters *viz.*, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum cholesterol and total protein contents[15-18].

Estimation of liver biochemical parameters: Lipid peroxidation *i.e.* thiobarbituric acid reactive substances (TBARS) was estimated by the reported method and expressed as mM/100 g of tissue[19]. Reduced glutathione (GSH) was determined by the standard method and was expressed as mg/100 g of tissue[20]. Catalase (CAT) was assayed according to the previously described method and expressed as μmoles of H_2O_2 consumed/min/mg of liver tissue[21].

Statistical analysis: The experimental results were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's post hoc test of significance. p values of < 0.001 were considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity: The oral LD_{50} value of the petroleum ether extract of *Dregea volubilis* fruit (PEDV) in mice was 900 mg/kg body weight.

Fasting blood glucose (FBG) level: The fasting blood glucose levels of normal, diabetic and treated rats are summarized in Table-1. Streptozotocin at the dose of 65 mg/kg produced marked hyperglycemia as evident from significant ($p < 0.001$) elevation in fasting blood glucose level in streptozotocin control group as compared to normal control group. Administration of petroleum ether extract of *Dregea volubilis* in streptozotocin-induced diabetic rats at the doses of 100 and 200 mg/kg b.w. produced significant ($p < 0.001$) and dose dependent fall in blood glucose levels when compared with the streptozotocin-control group. The fasting blood glucose reducing effect by petroleum ether extract of *Dregea volubilis* at the dose of 200 mg/kg b.w. was found to be comparable to that of the reference drug glibenclamide (0.5 mg/kg b.w.).

Body weight, liver and kidney weight: The body weight, liver and kidney weights of rats from streptozotocin control group (after 15 days) were significantly ($p < 0.001$) decreased when compared with normal control group. Petroleum ether extract of *Dregea volubilis* at 100 and 200 mg/kg b.w. signifi-

TABLE-1
INFLUENCE OF PEDV ON FASTING BLOOD GLUCOSE LEVEL (mg/dL) IN NORMAL AND DIABETIC RATS

Group	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal saline)	5 mL/kg	77.85 ± 2.1	76.32 ± 4.2	75.92 ± 3.6	74.72 ± 2.9
II (streptozotocin)	65 mg/kg	277.54 ± 9.2*	281.95 ± 10.5*	286.73 ± 11.4*	294.72 ± 9.8*
III (streptozotocin + PEDV)	100 mg/kg	263.36 ± 11.9	115.65 ± 7.8**	99.45 ± 1.6**	89.73 ± 1.8**
IV (streptozotocin + PEDV)	200 mg/kg	264.76 ± 16.5	90.29 ± 5.4**	80.58 ± 3.2**	72.91 ± 2.9**
V (streptozotocin + Gliben.)	0.5 mg/kg	278.53 ± 17.3	94.45 ± 3.8**	81.49 ± 1.5**	71.91 ± 2.4**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with normal control and **p < 0.001 compared with streptozotocin control group. Gliben: Glibenclamide.

TABLE-2
INFLUENCE OF PEDV ON BODY WEIGHT, KIDNEY WEIGHT AND LIVER WEIGHT IN NORMAL AND DIABETIC RATS

Group	Dose	Initial body wt (g)	Final body wt (g)	Final liver wt (g)	Final kidney wt (g)
I (Normal saline)	5 mL/kg	178.76 ± 7.8	184.54 ± 5.2	6.75 ± 2.9	1.33.6 ± 1.3
II (streptozotocin)	65 mg/kg	175.68 ± 7.2	149.44 ± 4.5*	3.15 ± 2.3*	0.83 ± 1.1*
III (streptozotocin + PEDV)	100 mg/kg	166.54 ± 4.2	150.52 ± 2.9**	5.81 ± 3.3**	1.11 ± 1.5**
IV (streptozotocin + PEDV)	200 mg/kg	168.41 ± 5.2	153.94 ± 1.8**	5.99 ± 3.6**	1.27 ± 1.2**
V (streptozotocin + Gliben.)	0.5 mg/kg	177.53 ± 4.5	166.76 ± 3.3**	6.36 ± 3.1**	1.28 ± 1.6**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with normal control and **p < 0.001 compared with streptozotocin control group. Gliben: Glibenclamide.

TABLE-3
INFLUENCE OF PEDV ON SERUM BIOCHEMICAL PARAMETERS IN NORMAL AND DIABETIC RATS

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	Total protein (g/dL)	SALP (U/L)	Cholesterol (mg/dL)
I (Normal saline)	5 mL/kg	21.15 ± 4.9	24.8 ± 3.8	7.28 ± 1.1	167.1 ± 13.2	153.63 ± 9.6
II (streptozotocin)	65 mg/kg	38.5 ± 5.5*	41.5 ± 4.5*	4.62 ± 0.5*	237.5 ± 11.8*	213.6 ± 13.8*
III (streptozotocin + PEDV)	100 mg/kg	29.9 ± 3.6**	28.6 ± 3.7**	6.08 ± 2.9**	215.9 ± 13.3**	194.8 ± 12.8**
IV(streptozotocin + PEDV)	200 mg/kg	23.3 ± 3.3**	24.8 ± 3.3**	6.77 ± 3.2**	176.6 ± 15.6**	166.5 ± 11.2**
V (streptozotocin + Gliben.)	0.5 mg/kg	21.5 ± 2.9**	24.8 ± 3.7**	7.15 ± 3.3**	171.7 ± 9.8**	160.7 ± 10.5**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with normal control and **p < 0.001 compared with streptozotocin control group. Gliben: Glibenclamide.

TABLE-4
INFLUENCE OF PEDV ON LIVER BIOCHEMICAL PARAMETERS IN NORMAL AND DIABETIC RATS

Group	Dose	TBARS (mM/100 g of wet liver tissue)	GSH (mg/ 100 g of wet liver tissue)	CAT (μmoles of H_2O_2 consumed/min/ mg of wet liver tissue)
I (Normal saline)	5 mL/kg	1.14 ± 0.5	46.54 ± 1.8	86.67 ± 6.3
II (streptozotocin)	65 mg/kg	1.95 ± 0.5*	27.32 ± 1.3*	42.72 ± 3.6*
III (streptozotocin + PEDV)	100 mg/kg	1.25 ± 0.9**	33.66 ± 1.1**	71.54 ± 4.5**
IV (streptozotocin + PEDV)	200 mg/kg	1.15 ± 0.4**	38.49 ± 1.5**	79.32 ± 3.6**
V (streptozotocin + Gliben.)	0.5 mg/kg	1.15 ± 0.2**	40.59 ± 1.3**	81.54 ± 7.1**

Values are expressed as mean ± SEM (*n* = 6); *p < 0.001 compared with normal control and **p < 0.001 compared with streptozotocin control group. Gliben: Glibenclamide.

cantly (p < 0.001) increased the body weight, liver and kidney weights towards normal in a dose dependent manner as compared to streptozotocin control (Table-2).

Serum biochemical parameters: Biochemical parameters like SGOT, SGPT, SALP and serum cholesterol in the streptozotocin control group were significantly (p < 0.001) elevated as compared to the normal control group. Treatment with petroleum ether extract of *Dregea volubilis* at the dose of 100 and 200 mg/kg b.w. significantly (p < 0.001) brought the SGOT, SGPT, SALP and serum cholesterol levels towards the normal values in a dose dependent manner. The total protein was found to be significantly decreased in the streptozotocin control group as compared with the normal control group (p < 0.001). Administration of petroleum ether extract of *Dregea volubilis* in diabetic animals significantly (p < 0.001) increased the total protein content as compared with the streptozotocin control group (Table-3).

Liver biochemical parameters: The levels of TBARS were significantly (p < 0.001) increased in streptozotocin control animals as compared to normal control group. Treatment with petroleum ether extract of *Dregea volubilis* at 100 and 200 mg/kg b.w. significantly (p < 0.001) reduced the TBARS levels when compared with streptozotocin control animals in dose related manner. The level of reduced glutathione (GSH) was significantly (p < 0.001) depleted in streptozotocin control group as compared with normal control group. Reduced GSH level was found to be significantly and dose dependently (p < 0.001) elevated towards normal level on administration of petroleum ether extract of *Dregea volubilis* as compared with streptozotocin control group. There was significant (p < 0.001) reduction in catalase activity in streptozotocin control group compared with normal group. Administration of PEEDV significantly (p < 0.001) recovered CAT activity towards normal when compared with streptozotocin control animals (Table-4).

The present work was aimed to study the antidiabetic activity of petroleum ether extract of *Dregea volubilis* fruit (PEDV) in streptozotocin-induced diabetic rats. The results of this study revealed that petroleum ether extract of *Dregea volubilis* at the doses of 100 and 200 mg/kg b.w. significantly normalized the elevated blood glucose level and restored serum and liver biochemical parameters towards normal values.

Streptozotocin (STZ) is an antibiotic obtained from streptomyces achromogenes, streptozotocin possess diabetogenic properties mediated by pancreatic β-cell destruction, hence this compound has been widely used to induce diabetes mellitus in experimental animals[22]. Once streptozotocin enters into cells, it undergoes spontaneous decomposition to form an isocyanate and a methyldiazohydroxide compound. Isocyanate and methyldiazohydroxide compound cause intra molecular carboxylation and alkylation of cellular components respectively[23]. The DNA damage of ß cells of pancreas is mainly by alkylation with carbonium ion produced by methyldiazohydroxide[24]. streptozotocin is not only damaging to the pancreatic β-cells but also to hepatocytes, nephrons and cardiomyocytes.

Hyperglycemia was observed after 3 days of streptozotocin-induction. Treatment with petroleum ether extract of *Dregea volubilis* in streptozotocin-induced diabetic rats, at the both test doses started reducing fasting blood glucose levels after 5 days and made them normoglycemic after 15 days. The antidiabetic effect of petroleum ether extract of *Dregea volubilis* at 200 mg/kg b.w. dose was found to be comparable to that the effect exerted by the reference drug, glibenclamide at the dose of 0.5 mg/kg b.w. petroleum ether extract of *Dregea volubilis* also showed marked effect in controlling the loss of body weight, liver and kidney weights of diabetic rats.

Elevation of serum biomarker enzymes such as SGOT, SGPT and SALP was observed in diabetic rats indicating impaired liver functions which may be due to hepatic damage. The decreased total protein content in streptozotocin-induced animals also substantiated the hepatic damage by streptozotocin. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activities[25]. 15 days of treatment with petroleum ether extract of *Dregea volubilis* restored all the above mentioned parameters towards the normal levels in a dose dependent manner. It is well known that in uncontrolled diabetes mellitus, there is an increase in total cholesterol in blood which may contribute to coronary artery diseases[26]. In the present study the elevated serum cholesterol level in diabetic rats was normalized after treatment with petroleum ether extract of *Dregea volubilis*. This suggests that the extract may inhibit the pathway of cholesterol synthesis in diabetic rats.

Oxidative stress in diabetes mellitus has been shown to co-exist with a reduction in the endogenous antioxidant status[27]. Several evidences suggest that streptozotocin induces oxidative stress. Oxidative stress is caused by a relative overproduction of reactive oxygen species (ROS). Reactive oxygen species results in lipid peroxidation and subsequently increased in TBARS levels leading to degradation of cellular macromolecules. A marked increase in the concentration of TBARS in streptozotocin -induced diabetic rats indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent overproduction of reactive oxygen species. Lipid peroxidation is usually measured in terms of TBARS as a biomarker of oxidative stress[28]. Treatment with petroleum ether extract of *Dregea volubilis* inhibited hepatic lipid peroxidation in diabetic rats as revealed by reduction TBARS levels towards normal levels. This indicated the inhibition in free radicals (ROS) generation in streptozotocin-induced diabetic rats.

Glutathione plays an important role in the endogenous non-enzymatic antioxidant system. Primarily it acts as reducing agent and detoxifies hydrogen peroxide in presence of an enzyme glutathione peroxidase[29]. The depleted reduced glutathione (GSH) may be due to reduction in GSH synthesis or degradation of GSH by oxidative stress in streptozotocin-induced hyperglycemic animals[30]. Petroleum ether extract of *Dregea volubilis* treatment significantly elevated the reduced hepatic glutathione level towards normal in diabetic rats. The results showed that the antidiabetic activity of petroleum ether extract of *Dregea volubilis* was accompanied with the enhancement in non-enzymatic antioxidant protection. These findings suggest that the petroleum ether extract of *Dregea volubilis* may exert its antidiabetic effect through the enhancement of cellular antioxidant system.

Enzymatic antioxidant mechanisms play an important role in the elimination of free radicals (ROS). Catalase (CAT) is a haem containing enzyme catalyzing the detoxification of H_2O_2 to water and oxygen[31]. The inhibition of catalase activity as a result of streptozotocin-induced hyperglycemia was reported earlier and the similar findings were observed in our present study[32,33]. Petroleum ether extract of *Dregea volubilis* treatment significantly recovered the CAT activity towards normal in a dose dependent manner.

Conclusion

In the present study, administration of petroleum ether extract of *Dregea volubilis* to streptozotocin-induced diabetic rats demonstrated prominent reduction in blood sugar level, normalization of serum biochemical profiles comparing to streptozotocin control rats. Also petroleum ether extract of *Dregea volubilis* treatment resulted in significant modulation of lipid peroxidation, endogenous non-enzymatic (GSH) and enzymatic (CAT) antioxidant and detoxification systems. Therefore, it can be concluded that the methanol extract of *Dregea volubilis* fruit is remarkably effective against streptozotocin-induced diabetes in Wistar rats plausibly by virtue of its augmenting endogenous antioxidant mechanisms.

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