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Effect of various extracts of *Desmodium gangeticum* on Streptozotocin-nicotinamide induced type-2 diabetes

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ABSTRACT

The antidiabetic effect of various extracts of Desmodium gangeticum (D. gangeticum) was evaluated on normal and streptozotocin (STZ)-nicotinamide induced type-2 diabetic animals. Type-2 diabetes was induced in Wistar albino rats of either sex by the administration of STZ-nicotinamide (40, 110 mg/kg b.w., respectively) intraperitoneally. D. gangeticum (100 mg/kg b.w.) extracts in different solvents (viz. pet. ether, benzene, chloroform, acetone, ethanol and water) were administered to diabetic rats for 21 days. The effect of extracts on blood glucose, lipid profile (TC, TG, LDL-C and HDL-C) and body weight was studied in diabetic rats. D. gangeticum extracts significantly reduced the elevated blood glucose, TC, TG, LDL-C level. Reduced HDL-C level and body weight in diabetic animals were found to be elevated significantly by the D.gangeticum extracts. But among all the extracts aqueous extract exhibited the best antidiabetic, antihyperlipidemic activity and positive effect on weight of diabetic rats. The results of our study suggest that the aqueous extract of D.gangeticum possesses a promising effect on STZ-nicotinamide-induced type-2 diabetes.

Key words: *Desmodium gangeticum*, Diabetes, Antidiabetic activity, Type-2 diabetes, Streptozotocin-nicotinamide

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INTRODUCTION

Diabetes is a chronic disease that is relatively common throughout the world [1]**.** The term diabetes mellitus is used to refer to a metabolic disorder of multiple etiologies, in which chronic hyperglycemia is caused by defects in either the secretion or action of insulin or alterations to both of these processes. This results in disturbances in carbohydrate, fat and protein metabolism [2]. Free radicals may play an important role in the causation and complication of diabetes mellitus. The increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of diabetes [3]**.** It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide [4]**.**

The incidence of diabetes is increasing. Worldwide, it affects 230 million people of which 30 million are in India. It has been estimated that by the year 2025, the global incidence of diabetes would increase to 350 million [5]**.**

On a global level, Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population. The World Health Organization has predicted that the major burden will occur in the developing countries. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. The countries with the largest number of diabetic people are, and will be in the year 2025, India, China and United States [6, 7].

Management of diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands, the recombinant DNA approach to diabetes management originally considered as a panacea has faced several problems [4]**.** Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge [8].

Given a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes, systematic and intensive search in plants for new drugs to treat Type 2 diabetes mellitus seem to be of great utility. This approach seems likely to increase the chances for discovering new drugs for the management of Type 2 diabetes mellitus [4]**.** Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation.

D. gangeticum DC (Leguminosae) is a small perennial shrub growing throughout India [9]**.** This has been commonly used in indigenous system of medicine (Ayurveda) as bitter tonic, febrifuge, digestive, anti-catarrhal, anti-emetic, in inflammatory conditions of chest and in various other inflammatory conditions which are due to vata-disorders **[**10, 11]. This plant has been used in Ayurveda for the treatment of various diseases like typhoid fever, urinary discharges, piles, inflammations, asthma, bronchitis, vomiting, dysentery and hemicrania [12]**.** It is used in 'Ayurvedic' preparations like 'Dashmoolarishta' and 'Dashmoolakwaath' for the post-natal care to avoid secondary complications [13].

The aqueous extract of this plant has been reported to show severe antiwrithing activity, moderate central nervous system (CNS) depressant activity [14] and antileishmanial activity [15]. Gangetin, a pterocarpnoid from *D. gangeticum* has been shown to possess anti-inflammatory and analgesic activity [16]. Total alkaloids of this species showed anticholinesterase, smooth muscle stimulant, CNS stimulant and depressant responses [17]. Chemical studies on the *D. gangeticum* revealed the presence of alkaloids, pterocarpnoids, flavonoids and isoflavonoid glycosides [10, 18].

However, despite the various bioactive phytochemical and diverse medicinal activities attributed to this plant, no biochemical studies have been carried out to shed light on the role of this plant in Type-2 diabetes. In the light of the above, the current study was undertaken to investigate its role on blood glucose, lipid profile (TG, TC, LDL-C and HDL-C) and body weight of type-2 diabetic animals.

MATERIALS AND METHODS

Animals

Albino Wistar rats of either sex, weighing (150-200 g), were obtained from Animal House, Shri Guru Ram Rai Institute of Technology and Science, Dehradun (Uttarakhand). The animals were kept under standard conditions of 12:12 h light and dark cycle in polypropylene cages and fed with standard laboratory diet and water *ad libitum*. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. The study was approved by Institutional Animal Ethical Committee (IAEC), Shri Guru Ram Rai Institute of Technology and Science, Dehradun (Uttarakhand). *(Regd. No. 264/CPCSEA)*

Chemicals

Streptozotocin was purchased from Sigma-Aldrich (St Louis, MO, USA). Standard drug, Glimepiride was obtained as a gift sample from Ranbaxy lab, Poanta Sahib, (H.P.), India. For the estimation of lipid profile, diagnostic kit was purchased from Reckon Diagnostic Pvt. Ltd. All the chemicals used in the experiment were of analytical grade and obtained from Himedia laboratories Pvt. Ltd. Mumbai, India.

Preparation of plant extract

Aerial parts of *D. gangeticum* were collected during the month of July-August from local herbal garden of Dehradun (Uttarakhand). The plant was taxonomically identified and authenticated at the Forest Research Institute (FRI), Dehradun. A voucher specimen (No.157028) was deposited in the Botany division of FRI, Dehradun. Fresh aerial parts of *D. gangeticum* were washed and shade dried than coarsely powdered in a grinder. Powder dried plant were extracted with solvents of increasing polarity (petroleum ether, benzene, chloroform, acetone and alcohol) by the

method of continuous hot extraction. Aqueous extract was prepared by the process of maceration. Each extract were concentrated, dried *in vacuo* and the residue stored in a desiccators for further use.

Experimental induction of diabetes [19].
Type-2 diabetes mellitus or Non- insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of STZ (40 mg/kg b.w.), 15 min later; the rats were given the intraperitoneal administration of nicotinamide (NAD) (110 mg/kg b.w.). STZ was dissolved in citrate buffer (pH 4.5) and NAD was dissolved in normal physiological saline. During the first 24 h of diabetes induction, STZ-treated animals were allowed to drink 5% glucose solution to overcome drug-induced hypoglycemia. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 hrs, then on day 7 and 14 after injection. Animals showing fasting blood glucose higher than 230 mg/dl were considered as diabetic and used for the further study.

Acute toxicity test [20]
Acute oral toxicity study for the test extracts of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Healthy albino Wistar rats (200–250 g) were used for this study. Animals were fasted (food but not water was withheld overnight) prior to dosing. The fasted body weight of each animal was determined, and the dose was calculated according to the body weight.

Limit test at 2000 mg/kg
The drug was administered in the dose of 2000 mg/kg body weight orally to one animal. A total of six animals were tested. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days. No animal died. Therefore, the LD_{50} is greater than 2000 mg/kg.

An investigation with $1/20^{th}$, $1/10^{th}$, and $1/5^{th}$ of 2000 mg/ kg, i.e. 100, 200, and 400 mg was done in pre-screening. For all the extracts, 100 mg/kg was found to be effective against diabetes but with different efficacy, hence this dose was used for all the extracts in final screening.

Experimental design
Animal were divided into nine groups having six rats in each group and all groups of animal were received treatment for 21 days. **Group- 1:** Normal control, **Group-2:** Diabetic control, **Group-3:** Diabetic animal + Glimepiride (10 mg/kg); **Group-4:** Diabetic animal + Petroleum ether extract of *D. gangeticum* (DG) (100mg/kg); **Group-5:** Diabetic animal + Benzene extract of DG (100 mg/kg); **Group-6:** Diabetic animal + Chloroform extract of DG (100 mg/kg); **Group-7:** Diabetic animal + Acetone extract of DG (100 mg/kg); **Group-8:** Diabetic animal + Ethanolic extract of DG (100 mg/kg); **Group-9:** Diabetic animal + Aqueous extract of DG (100 mg/kg).

Collection of blood sample
Blood was collected from retro-orbital plexuses and serum samples were analyzed for blood glucose and lipid profile. Fasting blood glucose measurements was done on 1^{st}, 7^{th}, 14^{th}, 21^{st} day of the study by GOD-POD method. On 21^{st} day of study, body weight and lipid profile (TC, TG, HDL and LDL) were also estimated.

Statistical Analysis
All the results were expressed as the mean ± Standard error mean (SEM). Data was analyzed by using two way ANOVA followed by tukey's multiple comparison as post-hoc test. The limit of statistical significance was set at $P<0.05$.

RESULTS

Acute toxicity studies
Acute toxicity studies conducted revealed that the administration of all the extract (up to a dose of 2000 mg/kg) of *D.gangeticum* did not produce significant changes in behavior of the animals. No death was observed up to the dose of 2000 mg/kg b.w. The rats were physically active. These effects were observed during the experimental period (14 days). The results showed that in single dose the plant extracts had no adverse effect, indicating that the medium

lethal dose (LD_{50}) could be greater than 2000 mg/kg body weight in rats. In acute toxicity study, no toxic symptoms were observed up to dose of 2 g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study.

Blood glucose level

The present study demonstrated the antidiabetic effect of aerial part extracts of *D. gangeticum* on blood glucose profile, body weight and lipid profile of type- 2 diabetes in wistar rats. The study showed the fasting plasma glucose (FPG) values, before and after treatment for 21 days in normal, diabetic untreated and diabetic treated with standard drug glimepiride and petroleum ether, benzene, chloroform, acetone, ethanol and aqueous extracts of *D. gangeticum*. The fasting glucose values remained more or less the same in normal group i.e. 92.83± 4.045 before and 112.0±3.416 mg/dl after 21 days. However after treatment with 10mg/kg of glimepiride and 100mg/kg of petroleum ether, benzene, chloroform, acetone, ethanol and aqueous extracts of *D. gangeticum* showed high initial FPG values (213.5±9.946, 269.83 ± 6.879, 274.33 ± 7.728, 269.5 ± 8.875, 264 ± 9.937, 246.33 ± 10.137and 238 ± 7.797 respectively), which came back close to normal level (106.83±4.324,128±1.579, 146.66 ± 8.875, 133.33 ± 6.667, 128.33 ± 6.009, 122.16 ± 7.530, and 109 ± 5.250 respectively) after 21 days. The blood glucose was elevated significantly in diabetic rats as compared with normal control rats. In diabetic rats, treatment with glimepiride and DG extracts (100 mg/kg) lowered the blood glucose significantly as compared with diabetic control. Among all the extracts, aqueous extract showed the best antidiabetic effect. Results of effect of plant extracts on fasting blood glucose level is presented in **Table-1.**

Table No. 1 Effect of treatment for 21 days with aerial parts extracts of *D. gangeticum* on fasting plasma glucose level in type -2 diabetic animals

Fasting Plasma Glucose (mg/dl) mean ± SEM				
Groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal control	92.83± 4.045	95.67±4.364	105.83±3.911	112.0±3.416
Diabetic control	231.66± 8.724$^{a\#}$	291 ± 5.426 ^{a*}	298.33 ± 8.720 ^{a*}	305 ± 13.784 ^{a*}
Diabetic + Glimepride	213.5±9.946^{a†}	182.67±7.632^{b‡}	145.67±6.702$^{b\#}$	106.83±4.324^{b*}
Diabetic +Pet. Ether extract of DG	269.83 ± 6.879^{a*}	203.53 ± 7.855^{a‡}	146.66 ± 7.845 $^{b\#}$	128 ± 11.579 ^{b*}
Diabetic +Benzene extract of DG	274.33 ± 7.728^{a*}	254.667 ± 14.013 ^{a*}	209.66 ± 7.648^{a‡}	146.66 ± 8.875 ^{b*}
Diabetic + Chloroform extract of DG	269.5 ± 8.875^{a*}	217 ± 6.11^{a†}	191.66 ±11.664^{b‡}	133.33 ± 6.667 ^{b*}
Diabetic +Acetone extract of DG	264 ± 9.937^{a*}	218.33 ± 8.720^{a†}	156.66 ± 10.537 $^{b\#}$	128.33 ± 6.009 ^{b*}
Diabetic + Ethanolic extract of DG	246.33 ± 10.137$^{a\#}$	201.67 ± 10.243^{a‡}	149.66 ± 7.528 $^{b\#}$	122.16 ± 7.530 ^{b*}
Diabetic + Aqueous extract of DG	238 ± 7.797$^{a\#}$	185.66 ± 9.630^{b‡}	139.33 ± 10.735^{b*}	109 ± 5.250 ^{b*}

*Fasting blood glucose values (mg/dl) are the mean ± SEM of six rats; [a] significant compared with normal control group and [b] significant compared with diabetic control group, *= p<0.0001, [≠] = p< 0.001,[†]= p< 0.01,[‡]= p<0 .05*

Effect on serum lipid profile

The results of the serum lipid profile showed that in streptozotocin-nicotinamide induced Type-2 diabetic animal there was not only hyperglycemia but also hyperlipidemia in which significant increase in serum triglyceride, total cholesterol, LDL-C and significant reduction in HDL-C was observed compared to normal control.

Table No. 2 Effect of treatments for 21 days with aerial part extracts of *D.gangeticum* on lipid profile of Type-2 diabetic animals

Groups	**TC(mg/dl)**	**HDL(mg/dl)**	**TG(mg/dl)**	**LDL (mg/dl)**
Normal control	141.46±6.042	46.62 ± 2.266	106.66 ± 5.04	73.51 ± 6.012
Diabetic control	229.73±9.043^{a*}	30.36 ±2.62a†	195.53 ± 9.87^{a}*	150.28 ± 12.37 ^{a*}
Diabetic +Glimepiride	180.94±3.466 $^{a\dagger\dagger b\dagger}$	42.91 ± 1.874	124.89 ± 8.087^{b*}	113.06 ± 5.781^{a††}
Diabetic +Pet. ether extract of DG	202.34±1.539 ^{a*}	43.89 ± 2.009^{b††}	175.16 ± 11.685 $^{a*c\#}$	123.43 ± 12.455^{a†}
Diabetic +Benzene extract of DG	208.24± 2.519 ^{a*}	46.78 ± 3.817^{b†}	177.72 ± 12.694$^{a*c\#}$	125.91 ± 10.454 ^{a†}
Diabetic + chloroform extract of DG	218.04 ±13.721^{a*}	44.78 ± 3.078^{b††}	186.87 ± 9.222 ^{a*b*}	133.87 ± 9.582$^{a\#}$
Diabetic +Acetone extract of DG	207.19 ±6.989 ^{a*}	42.49 ± 1.319	185.89 ± 6.536^{a*b*}	127.54 ± 8.536^{a††}
Diabetic + Ethanolic extract of DG	197.43± 15.081 $^{a\neq}$	51.39 ± 1.343$^{b\#}$	128.69 ± 11.219$^{a\dagger b\dagger}$	93.90 ± 12.219$^{b\#}$
Diabetic + Aqueous extract of DG	161.29 ±10.484 ^{b*}	52.84 ± 2.552^{b*}	120.90± 3.633^{b*}	84.2±4.651^{b}*

*Lipid profile (mg/dl) is the mean ±SEM of six rats; [a] significant compared with normal control group and [b] significant compared with diabetic control group, *= p<0.0001, [≠] = p< 0.001,[†]= p< 0.01,[‡]= p<0 .05*

Treatment of diabetic rats with *D.gangeticum* extracts for 21 days resulted in decrease of serum lipid profile as compared to diabetic control and the value came down significantly close to normal level when compared to normal control group. A significant reduction in TC was observed in animals treated with aqueous extract as compared to

diabetic control (p<0.0001). The effect of standard drug (p<0.01) in reducing TC was less significant than aqueous extract. The level of TG and LDL-C was found to be reduced significantly in diabetic animals treated with aqueous extract followed by ethanolic extract as compared to diabetic control group (p< 0.0001 and 0.01 respectively). HDL-C decreased significantly in diabetic animals when compared to normal control group **(Table- 2)**.

Effect on Body weight

A significant decrease in body weight was observed in diabetic untreated group (p<0.01) as compared to normal control group. Among the various extracts, administration of aqueous extract exhibited the significant increase (p<0.0001) in body weight after 21 days of treatment followed by ethanolic extract (p<0.05) The results of the study also showed that the effect of aqueous extract (p<0.0001) on body weight was more significant than standard drug (p<0.01). Results are shown in **Table -3**.

Table No. 3 Effect of treatment for 21 days with *D.gangeticum* on body weight in type -2 diabetic animals

S. No.	Groups	Day 1	Day 21
1.	**Normal control**	145 ± 3.651	158.54 ± 4.944
2.	**Diabetic control**	150.85 ± 7.303	116.55 ± 6.667^{a†}
3.	**Diabetic + Glimepiride**	147.33 ± 6.146	159 ± 5.627^{b†}
4.	**Diabetic + Pet. ether extract of DG**	120 ± 5.774	134.78 ± 5.164
5.	**Diabetic + Benzene extract of DG**	168.23 ± 12.649	145 ± 10.878
6.	**Diabetic + Chloroform of DG**	155.45 ± 7.638	146 ± 6.667
7.	**Diabetic + Acetone extract of DG**	140 ± 6.325	150 ± 6.325
8.	**Diabetic + Ethanolic extract of DG**	130.66 ± 6.009	136.67 ± 7.149^{b††}
9.	**Diabetic + Aqueous extract of DG**	148.33 ± 7.032	168.45 ± 5.774^{b*}

*Body weight (gm) is the mean ±SEM of six rats; [a] significant compared with normal control group and [b] significant compared with diabetic control group, *= p<0.0001, ≠ = p< 0.001,†= p< 0.01,‡= p<0 .05*

DISCUSSION

Diabetes is now recognized as one of the killer disease with increasing the incidence over world- wide. Oral hypoglycemic agents especially the sulphonylureas and biguanides have been commonly used for the management of diabetes, especially the Type -2, in spite of the associated adverse effects. Attention is now focused on the use of plants and herbal remedies that would be devoid of serious side effects encountered with sulphonylureas and biguanides as alternative in the treatment of diabetes.

The single high dose of streptozotocin (STZ) injection can produce Type-1 diabetes by destroying the β-cells of the pancreas [21] in adult rats but when STZ injected in mild dose in rats develop Type-2 diabetes in the adult rats [22]. A new animal model of Type-2 diabetes has been produced by combination of STZ and nicotinamide administration in adult rats [23]. Nicotinamide has antioxidant property, exerts protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic β-cell mass producing Type-2 diabetes [24].

Significant increase in glucose level demonstrated the induction of diabetes by STZ-nicotinamide administration in experimental animals. The observed increase in the blood glucose was due to the abnormalities in pancreatic β-cell and thus affects the insulin secretion [25, 26]. By screening the various extracts of *D.gangeticum* for antidiabetic activity, most significant reduction in blood glucose level was observed with aqueous extract (p<0.0001) followed by pet. ether extract, acetone extract, ethanol extract (p<0.001) and chloroform extract (p<0.05) as compared to diabetic control group. On the basis of onset of antidiabetic activity aqueous extract has shown significant reduction in fasting blood glucose level by the 7th day of treatment whereas in other extract treated groups onset of antidiabetic activity was observed by the 14th day. Thereby, our study suggest that the aqueous extract of *D. gangeticum* has the most promising effect on blood glucose level of Type-2 diabetic animals.

The reduction in blood glucose level by extracts of *D.gangeticum* could possibly due to insulin like effect of the extract on peripheral tissues, either by promoting glucose uptake and metabolizing, or by inhibiting hepatic gluconeogenesis [27]. A significant effect on serum lipid profile (TC, TG, LDL and HDL) was observed with diabetic rats than normal control group which is often related with hyperlipidemia, a common complication of diabetes mellitus. Insulin plays an important role in metabolism of lipids. Its deficiency results in inactivation of

lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood and resulted into increased level of serum lipids [28].

The results of the study showed that aqueous extract of *D.gangeticum* ($p<0.0001$) reduces the TG, TC and LDL-C level significantly in treated diabetic animals compared to diabetic control group. Marked decrease in the level of TG and LDL-C was also observed with ethanol extract ($p<0.01$) of *D. gangeticum* in diabetic animals as compared to diabetic control.

HDL cholesterol (HDL-C) has an important role in the prevention of atherosclerosis by transporting the cholesterol from peripheral tissues to liver for excretion. Aqueous extract of *D. gangeticum* followed by ethanol extract reduces the HDL-C level significantly in diabetic rats compared to diabetic control group ($p<0.0001$ and 0.001 respectively). Increased HDL-C is associated with reduction in coronary risk. The results of the study indicates that the plant may possess insulin like activity which will be helpful to reduce the incidence of lipid born complications and thus reduces the CVS risk factors.

The induction of diabetes with STZ-nicotinamide is associated with the reduction in body weight due to increased muscle wasting or loss of muscle protein due to hyperglycemia [29, 30]. In our study, diabetic animal treated with aqueous extract of *D.gangeticum* results in significant increase in body weight indicating the proper utilization of glucose in animals.

In conclusion, the result of the study show that the aqueous extract of aerial part of *D. gangeticun* exhibited the best antidiabetic and hyperlipidemic effect in Type-2 diabetic model by reducing the fasting blood glucose level and improving the lipid profile. This confirmation justifies its use in ethnomedical medicine for the treatment of diabetes mellitus.

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