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ANTIDIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *DAUCUS CAROTA* ROOTS ON ALBINO WISTAR RAT

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ABSTRACT: Objective: The study's key objective is to evaluate the Antidiabetic activity of methanolic root extract of *Daucus carota* in normal, streptozotocin-induced Diabetic, and alloxan-induced diabetic rats. **Materials and Methods:** Albino Wistar rats were administered at a dose of streptozotocin 50mg/kg /day and alloxan monohydrate 150mg/kg/day i.p. for 30 days. Experimental rats were given *Daucus carota* root extract in two different doses of 200mg and 400mg/kg/day orally. **Results:** The Preliminary phytochemical investigation of *Daucus carota* shown that the presence of sterols, saponins, flavonoids, and triterpenoids. In this study, *Daucus carota* root extract was administered for 30 days and showed antidiabetic activity in STZ diabetic rats and alloxan-induced rats. **Conclusion:** The methanolic extract of *Daucus carota* roots also has potential antidiabetic activity in STZ-Nicotinamide diabetic rats. The extract also normalized the altered diabetic parameter *viz.* glycosylated hemoglobin, plasma cholesterol and glutathione. The decreased level of cholesterol indicates that the plant extract also has hypolipidemic activity.

INTRODUCTION: Diabetes mellitus (DM) is a major public health-related issue affecting more than 400 million people worldwide [1]. The various complications associated with DM include nephropathy, neuropathy, cardiovascular and renal complications, retinopathy, food-related disorders, etc. Type 1 DM and type 2 DM are the 2 types of DM. Type 1 DM is an autoimmune disorder that affects pancreatic cells which reduces or impairs the production of insulin, while type 2 DM is a result of impairment of pancreatic beta cells that hinder the individual's ability to use insulin [2].

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The major conventional classes of drugs for the treatment of hyperglycemia include sulfonylureas (enhance the release of insulin from pancreatic islets); biguanides (reduces hepatic glucose production); peroxisome proliferator-activated receptor-γ (PPARγ) agonists (boosts the action of insulin); α-glucosidase inhibitors (interferes with absorption of glucose in the gut) [3]. These classes of drugs are either administered as monotherapy or given in combination with other hypoglycaemics.

Severe hypoglycemia, weight gain, lower therapeutic efficacy owing to improper or ineffective dosage regimen, low potency and altered side effects due to drug metabolism and lack of target specificity, solubility, and permeability problems are the major drawbacks associated with the use of the above-mentioned conventional drugs [4]. Despite the advent of promising antihyperglycemic agents, the major

challenges in efficient diabetes treatment include optimizing the existing therapies to guarantee optimum and balanced glucose concentrations, as well as reducing long-term diabetes-related complications [5]. There are mainly two major types of diabetes mellitus. Several hormones maintain the homeostasis of glucose in the body. However, two hormones, namely, insulin and glucagon play a dominant role in regulating glucose homeostasis [6].

The most common type of diabetes is type 1 diabetes mellitus and Type 2 diabetes mellitus. Type 1 Diabetes Mellitus is an Insulin-dependent Diabetes Mellitus. Type 2 Diabetes Mellitus is Noninsulin dependent Diabetes Mellitus [7]. Type 1 Type 2 diabetes mellitus accounts for 90-95% of DM cases, with the remaining 5 to 10% being other types of DM, such as Type 1 Diabetes Mellitus, gestational diabetes, and other small specialized varieties that are rarely encountered [8]. To minimize the annual death rate from T1DM and T2DM, cost-effective treatment options are being searched substantially [9].

Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical issue in the world population. There is a need, and therefore for new compounds that may effectively reduce insulin resistance or potentiate insulin action in genetically diabetic or obese individuals alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes mellitus. There are most commonly employed oral hypoglycemic agents are sulfonylureas and biguanides. The research for a such drugs with a potential to reduce long-term complications of diabetes is, therefore of current interest. According to the WHO, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of diabetes mellitus have been reported. Medicinal plants are the most exclusive source of life-saving drugs for most of the world's population. In developing countries, 80% of the population uses traditional medicine for primary medical problems.

MATERIALS AND METHODS:

Collection of Plant Materials: *Daucus carota* roots purchased from the local market of Hisar were identified and authenticated by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, vide reference no. NISCAIR/RHMD/Consult/-2011-12/1996/04.

Preparation of Extract of *Daucus carota*: The roots were shade dried at 35±5°C and pulverized and used for carrying out the experimental work procedures. The air-dried crude drug (500 g) was pulverized and extracted with methanol using the Soxhlet apparatus for 16 h.

Methanol removal under pressure afforded a semi-solid mass yielding 21.5% w/w. The extract was further used for the evaluation of Phytochemical Screening and for evaluating antihyperglycemic activity

Preliminary Phytochemical Screening [10]: Preliminary phytochemical screening, each of the above extracts was tested for the presence of alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins, and amino acids as follows:

Tests for Alkaloids: About 500 mg of each of the dried extract was stirred with about 5 ml of dilute hydrochloric acid and filtered. The filtrate was tested with the following reagents:

Mayer's Reagent: Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added separately to each filtrate and observed for the formation of a white or cream-colored precipitate.

Dragendroff's Reagent: Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added separately to each filtrate and observed for the formation of an orange-yellow precipitate.

Hager's Reagent: Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added separately to each filtrate and observed for the formation of a yellow precipitate.

Wagner's Reagent: Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added separately to each filtrate and observed for the formation of a reddish-brown precipitate.

Tests for Carbohydrates:

Molisch's Test: A small amount of each extract was dissolved in ethanol, and two drops of a 20% w/v solution of α-napthol in ethanol were added to it. Now, about 1 ml of concentrated H_2SO_4 was slowly added along the sides of the test tube. The appearance of red-violet ring at the junction of the two layers indicated the presence of carbohydrates.

Fehling's Test: A small amount of each extract was dissolved in about 2 ml of distilled water and filtered. An equal amount of Fehling's solution was added to the filtrate, and the contents were boiled. The appearance of red brick precipitate confirmed the presence of reducing sugar.

Benedict Test: A small amount of each extract was dissolved in about 2 ml of distilled water and filtered. About 1 ml of Benedict solution was added to the filtrate. The contents were boiled and observed for the appearance of brick-red precipitate, confirming the presence of reducing sugar.

Tests for Glycoside:

Borntrager's Test: A small amount of each extract was hydrolyzed with dilute HCl for a few hours on a water bath. About 1.0 ml of benzene and 0.5 ml of dilute ammonia solution were added to the hydrolysate. The appearance of reddish-brown color at the junction of the two layers confirmed the presence of glycoside.

Keller-Killiani Test: A small portion of each extract was stirred with about 1 ml of glacial acetic acid, and after cooling, a few drops of ferric chloride solution were added.

The contents were then transferred to a test tube containing about 1 ml of concentrated H_2SO_4. After standing, a reddish brown layer acquiring bluish-green colour was observed as an indication of glycoside's presence.

Tests for Sterols:

Liebermann-Burchard's Test: A small amount of each extract dissolved separately in chloroform was added with a few drops of acetic anhydride followed by the addition of concentrated sulphuric acid drop-wise along the sides of the test tube and observed for the appearance of blue to blood red color as the indication of sterols.

Salkowski Reaction: About 2 ml of concentrated sulphuic acid was added to a few milligrams of each extract in their respective solvents and observed for the appearance of a yellow ring at the junction, which turned red after some time.

Hesse's Reaction: A few milligrams of each extract were separately dissolved in about 4 ml chloroform, and an equal quantity of concentrated H_2SO_4 was added along the sides of the test tube. The appearance of a pink-colored ring at the junction of two layers, which on shaking, diffused in both layers, confirmed the presence of sterols.

Hersch-Sohn's Reaction: About 2-3 ml of trichloroacetic acid was added to a small portion of each extract. The appearance of red to violet color on heating showed the presence of sterols.

Tests for Saponins:

Foam Test: About 1 ml of each extract (in the respective solvents) was diluted to 20 ml with distilled water and further shaken in a graduated cylinder for 15 minutes. The formation of about 1 cm thick foam layer confirmed saponin's presence.

Sodium Bicarbonate Test: A few drops of sodium bicarbonate solution were added and shaken to a small portion of each extract. The appearance of honeycomb-like frothing confirmed the presence of saponin.

Tests for Phenolic Compounds and Tannins:

Ferric Chloride Test: Small amounts of each extract were separately shaken with water and warmed. Now about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color.

Lead Acetate Test: A few milligrams of each extract were separately stirred with about 2 ml of distilled water and filtered. To the filtrate, a few drops of 10% w/v lead acetate solution was added and observed for the formation of white precipitates.

Tests for Amino Acids and Proteins

Millon's Test: A small amount of each extract was separately dissolved and filtered in about 5 ml of distilled water. To 2 ml of the filtrate, 5-6 drops of Millon's reagent (solution of mercury nitrate and nitrous acid) were added and observed for the

formation of red precipitate as an indication of the presence of proteins.

Ninhydrin Test: Lead acetate solution was separately added to each extract to precipitate the tannins. The filtrate was then spotted on a paper chromatogram, sprayed with ninhydrin reagent, and dried at 110 °C for 5 min. The appearance of violet spots confirmed the presence of free amino acids.

Tests for Flavonoids:

Shinoda/Pew Test: A few milligrams of each extract were separately shaken with ethanol in different test tubes. Now, small pieces of metallic magnesium or zinc were added, followed by 2 drops of concentrated HCl, and observed for the formation of pink color.

Ammonia Test: A small amount of each extract was separately shaken with ethanol. A filter paper strip was dipped in this alcoholic solution, made ammoniated, and observed for color change from white to orange.

Experimental Animals: The healthy albino Wistar rats of either sex, weighing 100–150 g, were used to evaluate antihyperglycemic activity after taking permission from 21th meeting of the Institutional Animal Ethics Committee (IAEC), held on 26th November 2012 at G.J.U. S and T, Hisar. They were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). The animals were housed under standard environmental conditions of temperature and humidity (25±0.50 °C) and (12 h light/dark cycle) was utilized for the studies. The animals were fed with a standard pellet diet and water *ad libitum*. The Institutional animal ethical committee (Guru Jambheshwar University of Science and Technology, Haryana, India) approved the experimental protocol and care of laboratory animals were taken as per the guidelines of CPCSEA, Ministry of Forest and Environment, Government of India (Registration number 0436).

Induction of Experimental Streptozotocin – Nicotinamide: The animal model of type-2 diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of 50mg/kg STZ. Hyperglycaemia was confirmed by the elevated blood glucose levels determined at 72 hour, then on 7th day of the injection. Only rats confirmed with permanent NIDDM were used in the antidiabetic study [11].

Induction of Experimental of Alloxan S. Diabetes: A freshly prepared solution of alloxan monohydrate (150mg/kg body weight) in normal saline was injected intraperitoneally to overnight fasted rats [12]. After 3 days, blood samples were collected retro-orbitally from the inner canthus of eyes using Micro Hematocrit Capillaries after at least 12 hours of fasting animals [13].

Blood was kept in fluoride tubes. It was then centrifuged at 6000 rpm for 15 minutes to obtain plasma. Glucose levels were estimated using the Glucose Oxidase method, and body weight was checked regularly up to stable hyperglycemia after 1 week of alloxan injection. The animals having marked hyperglycemia (Fasting blood glucose > 250 mg/dl) were selected for the study [14].

Experimental Design: To study the antihyperglycemic activity of extract of roots of *Daucus carota* using a type 2 diabetic model.

Each set was further divided into groups compromising of six animals per group, as listed below:

Animal Protocol for the Study of Antihyperglycemic Effect of *Daucus carota* extract:

Group 1: Normal control Rats.

Group 2 (Diabetic Control)*:* Animals were administered vehicle only.

Group 3: Diabetic animals were administered Glibenclamide (600 µg/kg; p. o.).

Group 4 and 5: Diabetic animals were treated with 250 mg/kg p.o. and 500 mg/kg p.o. *Daucus carota*, respectively.

Glucose Tolerance Test: After 29-day treatments, on the 30th day, a fasting blood sample was collected from all the groups in a heparinized micro-centrifuge tube from retro-orbital plexus. Blood samples were also collected at 0, 30, 60, 90, and 120 min after glucose administration at a concentration of 2 g/kg of body weight.

Biochemical Assay: The fasted animals were sacrificed by cervical decapitation on the 30th day of the first treatment. Trunk blood was collected in heparinised micro-centrifuged tubes and the plasma obtained by centrifugation at 5000 rpm for 5 min was used to determine plasma glucose, cholesterol, malondialdehyde (MDA), reduced glutathione. In contrast, whole blood was used for glycosylated hemoglobin.

Estimation of Serum Glucose and Cholesterol: Plasma cholesterol and glucose level were measured by commercially supplied biological kit Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method) respectively using Chem 5 Plus-V2 Auto-analyser (Erba Mannhein Germany) in serum sample prepared as above. Glucose and cholesterol values were calculated as mg/dl blood samples.

Estimation of Glycosylated Hemoglobin (Hb1Ac): Glycosylated hemoglobin was measured using a commercially supplied biological kit (Erba Diagnostic) in plasma sample prepared as above using Chem 5 Plus-V2 Auto-analyser (Erba Mannhein Germany). Values are expressed as the percent of total hemoglobin.

Estimation of Plasma Reduced Glutathione Level: 2.5 ml of blood plasma was mixed in a 10 ml test tube with 2 ml of distilled water and 0.5 ml of trichloroacetic acid.

The tubes were shaken intermittently for 10 to 15 minutes and centrifuged for 15 minutes at approximately 3000 rpm. The supernatant was mixed well with 2 ml of 0.4M Tris-EDTA buffer (pH 8.9) and 0.1 ml DTNB. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank [15].

Data Analysis: All the results were expressed as 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:

Preliminary Phytochemical Screening of *Daucus carota Roots*: Preliminary phytochemical study was carried out on the methanolic extract of *Daucus Carota* Linn roots. The results are shown in **Table 1.**

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF *DAUCUS CAROTA*

Sr. no.	Plant Constituents Test Reagents	Methanol extract
1.	**Alkaloids**	
	Mayer 'reagent	--
	Dragendroff's' reagent	--
	Hager' reagents	--
	Wagner's reagents	--
2.	**Glycosides**	
	Fehling solution	+
	Killer–Killani test	+
	Baljet test	--
	Borntrager test	+
	Legal test	--
3.	**Carbohydrates**	
	Molish's reagent	+
	Fehling solution	--
	Benedict test	--
4.	**Sterols**	
	Liebermann–Burchard test	+
	Salkowski test	+
5.	**Saponins**	
	Foam test	+
	Sodium bicarbonate test	+
	RBC Haemolysis test	--
6.	**Phenolics compounds & Tannins**	--
	Ferric chloride test	--
	Lead test	
7.	**Flavonoids**	
	Shinoda/Pew test	+
	Ammonia test	+
8.	**Proteins &Aminoacids**	
	Biuret test	+
	Ninhydrin reagent	+
9.	Triterpenes	
	Liebermann–Burchard test	+

+ve: present, -ve: absent.

Antihyperglycemic Effect of *Daucus Carota* Roots:

Effect of the Methanolic Extract of *Daucus carota* on Blood Glucose Levels in STZ-Induced Diabetic Rats: In this study, *Daucus carota* 250mg/kg bw p.o. to STZ diabetic rats for 30 days showed a fall in serum glucose level from 324.35 mg/dl to 177.33 mg/dl at 30th day when compared to 0 day value. At *Daucus carota* 500mg/kg bw p.o. there was a significant fall in serum glucose level from 290.63 mg/dl to 96.04 mg/dl on the 30th day ($p<0.001$).

TABLE 2: EFFECT OF THE METHANOLIC EXTRACT OF *DAUCUS CAROTA* ON BLOOD GLUCOSE LEVELS IN STZ- NICOTINAMIDE INDUCED DIABETIC RATS

S. no.	Group	Blood Glucose Levels (mg/dl)			
		Day 1	Day 10	Day 20	Day 30
1	Diabetic control	390.57±3.27	384.855±2.488	367.09±1.414	276.29±1.61
2	Glibenclamide	355.72±2.27*	254.21±1.457*	222.21±3.600*	136.36±2.567*
3	Methanolic 250	324.35±4.95*	213.31±1.497*	215.13±5.754*	177.33±3.461*
4	Methanolic 500	290.63±1.901*	189.57±1.319*	160.52±1.935*	96.04±1.013*

Values are presented as Mean±S.E.M..; n = 6 in each group. One way ANOVA followed by Dunnett's test, * denote p<0.01; Vs Diabetic control.

Effect of *Daucus carota* in Glucose Loaded Hyperglycaemic Rats: The effect of *Daucus carota* extract on plasma glucose level after glucose loading at 2g/kg bw orally to the normal, diabetic, and test animals was expressed in **Fig. 1**. The blood glucose level rises to a maximum in 30 min after glucose loading.

The extract-treated groups at both 200 mg/kg and 500 mg/kg bw showed a significant increase in the rate of clearance of glucose as compared to the untreated group. The extract-treated group showed a marked fall in glucose level in 30- to 120 min intervals. After 120 min the serum glucose level returns to normal value.

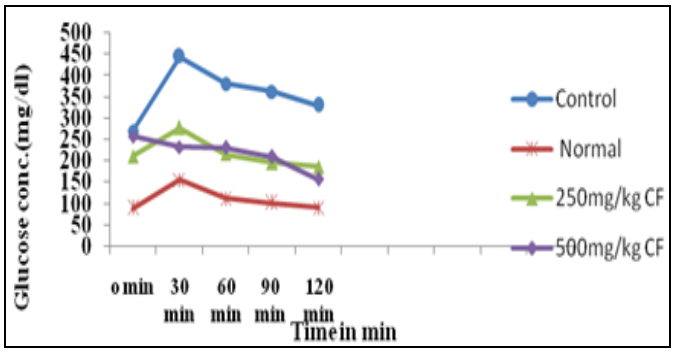

FIG. 1: EFFECT OF *DAUCUS CAROTA* IN GLUCOSE-LOADED HYPERGLYCAEMIC RATS. Values are presented as mean±S.E.M..; n = 6 in each group. One way ANOVA followed by Dunnett's test, * denote p<0.01; Vs Diabetic control.

Effect of *Daucus carota* on Glycosylated Hemoglobin: The data presented in **Fig. 2** indicated the effect of *Daucus carota* extract on glycosylated hemoglobin (HbA1c). The HbA1c data for the STZ diabetic group were significantly higher than the normal rats.

The administration of the standard drug significantly lowered the HbA1c in 30 days. The administration of extracts produced a similar effect to the standard drug, glibenclamide. Higher doses produced more reduction in HbA1c levels.

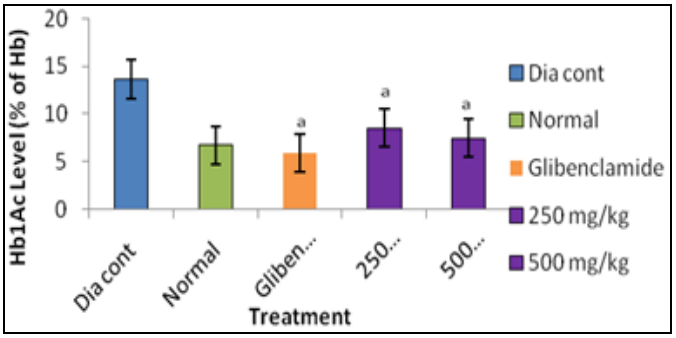

FIG. 2: EFFECT OF *DAUCUS CAROTA* ON GLYCOSYLATED HAEMOGLOBIN. Values are presented as mean±S.E.M..; n = 6 in each group. One-way ANOVA followed by Dunnett's test, a denote p<0.01; Vs control group.

Effect of *Daucus carota* on Total Cholesterol: The data depicted in **Fig. 3** indicated the effect of *Daucus carota* extract on total plasma cholesterol. Total plasma cholesterol was observed to be significantly (p<0.01) higher in diabetic rats compared to normal rats. The administration of the control drugs significantly lowered the total plasma cholesterol concentration. The administration of *Daucus carota* with 200 mg/kg, p. o. and 500 mg/kg, p. o. showed a significant (p < 0.01) reduction in total plasma cholesterol level.

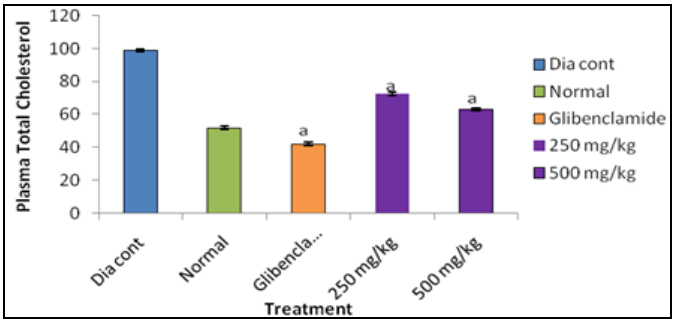

FIG. 3: EFFECT OF *DAUCUS CAROTA* ON TOTAL PLASMA CHOLESTEROL. Values are presented as Mean±S.E.M..; n = 6 in each group. One-way ANOVA followed by Dunnett's test a denote p<0.01; Vs control group

Effect of *Daucus carota* on Glutathione Level: The plasma glutathione (GSH) level was significantly decreased in STZ rats compared to

control animals **Fig. 4**. The glutathione level was returned to near normal range in STZ diabetic rats treated with *Daucus carota* and glibenclamide.

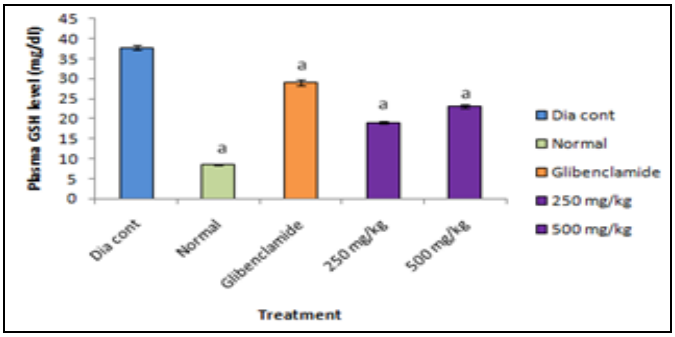

FIG. 4: EFFECT OF *DAUCUS CAROTA* ON PLASMA GSH LEVEL. Values are presented as Mean±S.E.M.; n = 6 in each group. One way ANOVA followed by Dunnett's test, a denote p<0.01; Vs. Diabetic control group, (GSH = Glutathione)

DISCUSSION: Diabetes mellitus is a metabolic disorder initially characterized by loss of glucose homeostasis with the disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. According to the WHO, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. A great number of medicinal plants used in the control of diabetes mellitus have been reported. A present study evaluated the antidiabetic potential of alcoholic extract in diabetic models with low-dose streptozotocin Wistar rats.

A common mechanism underlying hyperglycemia in Diabetes mellitus involves the overproduction and decreased glucose utilization by tissue. Present phytochemical studies of the plant revealed the presence of sterols, saponins, flavonoids and triterpenoids in *Daucus carota*. In the context of the present study antidiabetic and antioxidant activities of alcoholic extract of *Daucus carota* root were investigated on STZ diabetic rats. STZ diabetic model is widely employed for screening of type-2 antidiabetic activity. As previously discussed, Glibenclamide increases insulin secretion in STZ diabetic rats. 250mg/kg, 500mg/kg p.o. significantly reduced blood glucose level in nicotinamide -STZ-induced madel. The blood glucose level rises to a maximum in 30 min after glucose loading. The extract-treated groups at both 200 mg/kg and 500 mg/kg showed a significant increase in glucose clearance rate compared to the untreated group. The extract-treated group showed a marked fall in glucose level in 30 min to 120 min intervals. After 120 min the serum glucose level returns to normal value. The level of HbA1c and total cholesterol is reduced by *Daucus carota*. This cholesterol reducing activity may be explained based on improved glycemic control. *Daucus carota* significantly increased GSH level due to its free radical scavenging property

CONCLUSION: The methanolic extract of *Daucus carota* roots also has potential antidiabetic activity in STZ-Nicotinamide diabetic rats. The extract also normalized the altered diabetic parameter *viz.* glycosylated hemoglobin, plasma cholesterol, and glutathione. The decreased cholesterol level indicates that the plant extract also has hypolipidemic activity. The antihyperglycemic action of *Daucus carota* may be attributed to its involvement in improved insulin secretion, lowering glucose concentration, and insulin/ C-peptide response.

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CONFLICTS OF INTEREST: Nil

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