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RESEARCH ARTICLE

Investigation of Phytochemical, Pharmacological Activities of *Daucus Carota Subs P. Sativus* Extract on Streptozotocin Induced Diabetic Rats.

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ABSTRACT

The present study is to explore the methanolic extracts of leaves of *Daucus carota subsp sativus* and fractions were studied streptozotocin induced diabetic rats. The dried leaves were powder and extracted with methanol solvent by using hot continuous percolation (soxhelt) method. The alkaloids, carbohydrate, flavonoids, phenolic compounds, and tannins contents of methanolic extract and its fractions were also determined and correlated with its antidiabetic activity. The methanol extract and its fractions curtained for streptozotocin induced diabetic rats in this study gilbenclamide as cast-off for standard. Methanolic extract of *Daucus carota subsp sativus* revealed that LD_{50}>2000mg/kg, the biological dose was fixed at methanolic extract *Daucus carota subsp sativus* 200mg and 400mg of body weight for the extract. The methanolic extract of *Daucus carota subsp sativus* leaves result that revealed of alkaloids, carbohydrate, flavonoids, phenolic compounds, and tannins. The pharmacological activity of methanolic extract of *Daucus carota subsp sativus* leaves all values are expressed as statistically significant at a*= ($p<0.001$) for glucose tolerance and change in blood glucose level.

Keywords: *Daucus carota subsp sativus,* Solvent extraction, Streptozotocin and Glibenclamide, Oral glucose tolerance and *In-vivo* Anti-diabetic studies.

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INTRODUCTION

Diabetes mellitus is a cause for growing public health concern in both developed and developing countries. In many countries, it is now a leading cause of death, disability, and high health care cost. The WHO estimates that diabetes affects millions of people worldwide[1.] Developing nations, comprising most of the world's population may find responding to this resolution particularly challenging since many are now facing the double burden of both infectious and chronic, non-communicable disease [1].

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycaemic condition resulting from defects in insulin secretion, insulin action or both. Permanent neonatal diabetes is caused by glucokinase deficiency and is an inborn error of the glucose-insulin signalling pathway [2].

Type 1 diabetes and type 2 diabetes are heterogeneous diseases in which clinical presentation and disease progression may vary considerably3. The major clinical classes of glucose intolerance include insulin- dependent diabetes mellitus (IDDM or Type1) non-insulin- dependent diabetes mellitus (NIDDM or Type 2), malnutrition- related diabetes mellitus, Impaired glucose tolerance (IGT) and gestational diabetes (GDM). Type 1 diabetes is thanks to autoimmune β-cell destruction, usually resulting in absolute insulin deficiency. Type 2 Diabetes is due to a progressive loss of adequate b-cell insulin secretion frequently on the background of insulin resistance. Gestational diabetes occur diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation [3].

Diabetes has both acute and chronic complications. They are of variable speed of onset and severity; often adversely affect the individual's quality of life and they result in considerable premature disability and death. Acute metabolic complications include diabetic ketoacidosis, hypoglycaemia, and hyperosmolar coma. Chronic complications are nephropathy, retinopathy, neuropathy, and cardiovascular, cerebra-vascular, and peripheral vascular diseases [4].

Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. But these are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea [5]. Biological actions of the plants are related to chemical composition that are rich in phenolics, alkaloids, flavonoids, terpenoids, coumarins, and glycosides usually show positive effects. On the other hand, many conventional drugs for treatment of diabetes, such as metformin are secretagogues which have a plant origin [6]. The conventional drugs are used to treat diabetes by improving insulin sensitivity, increasing insulin production, and decreasing the amount of glucose in blood [7]. The adverse effect of drug treatment is not always satisfactory in maintaining normal levels of blood glucose and this view many medicinal plants have been provided a potential source of antidiabetic principle which are widely used for the treatment of diabetes mellitus in various traditional system of medicine worldwide and many of them are known to be effective against diabetes [8]. The hypoglycaemic effect of pharmacologically active component of plant decreases the effect on α-amylase and various direct and indirect effects of different blood parameters responsible for development of diabetes [9].

Diabetes and its consequences are the world's most serious disease. Various mechanisms, such as DPP-4 inhibitors, α-glucosidase inhibitors, α-amylase inhibitors, and insulin refunctioning, are involved in diabetes patients, the therapeutic agents, such as insulin and oral hypoglycemic medications. The current study's goal is to compare the pharmacological activity of herbal extracts to that of commercially available diabetic drugs. In this study, a healthy rat was given streptomycin to induce diabetes, and the pharmacological effect of *Daucus Carota Subs P.Sativus* extracts was compared to that of a commercially available medication.

MATERIALS AND METHODS

Collection and Authentication of plant material

The leaves of *Daucus carota subs p sativus* were carefully collected from the Ooty hills, Udhagamandalam and it were authenticated by Dr. C. Murugan (Scientist & Head Office) Botanical Survey of India, Coimbatore.

Drugs and chemicals

Streptozotocin (STZ) (LOBA Chemie, Mumbai, India) was purchased, preserved at 25ºC and used for this study. Glibenclamide is an oral antidiabetic preparation with an efficient hypoglycemic action. Diaonil (Glibenclamide) [10] manufactured by Aventis Pharma Ltd. Goa, India, was collected from market and preserved at room temperature. All other chemicals and reagents used in the study were of analytical grade.

Preparation of Plant Extract

The fresh leaves of *Daucus carota subs p sativus* were washed thoroughly with tap water and then in distilled water. The washed leaves were a shade drying to treat fungus until complete dryness of leaves. Then the leaves dried at room temperature and powdered by the electronic grinder until to get coarse powder. About 200g of dry powder was extracted in the various solvent by continuous hot percolation using Soxhlet apparatus. In extraction time keeps it temperature at 30 -40^{0}C.The extraction was continued for 7 days. After completion of solvent extract, it is distillation and concentrated to a dry mass by using rotary evaporator [11].

Animals

Swiss albino rats of Sprague–Dawley strain (200–250 g) of either sex obtained from animal house of our Institute were used. The animals were fed a standard pellet diet and water ad libitum. They were maintained in a controlled environment and temperature (22±5 ˚C with 12-h of light/dark cycle). All experimental protocols were approved by the Institutional Animal Ethical Committee (12/2009/CPCSEA) [12].

Acute toxicity study

Acute toxicity study of various solvent extract of *Daucus carota subsp sativus* was carried out in Swiss Albino rats of either sex (190-250g) according to OECD (Organization for Economic Cooperation and Development) guidelines No. 423. Extract at different doses up to 2000mg/kg p.o. was administered and the animals were observed for behavioural changes, toxicity, and mortality up to 48 hours [13-16].

Experimental oral glucose tolerance

The overnight fasted (18hr) normal rats were taken and divided into four groups consists of six animals. They were provided with drinking water only. Normal saline solution was administered to group I animals. Group II animals were received glibenclamide (3mg/kg,b.w) as a standard. *Daucus carota subsp sativus* methanol extract (200 and 400 mg/kg) was administered by oral route to group III and IV Glucose (2mg/kg) load was fed 30 minutes after the administration of extracts. Blood was withdrawn from tail vein under mild ether anaesthesia initial, 30,60 and 90minutes after glucose administration and glucose level were estimated using glucose strips and a glucometer (standard diagnostics Ltd). Blood glucose levels were noted and reported.

Experimental oral glucose tolerance

Female albino-Wistar rats weighing 150-250g were used in the present study. All rats were kept at room temperature of 22-25ºc in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for one week in order to adapt to the laboratory conditions in accordance with the recommendations for the proper care and use of laboratory animals. Fasting blood glucose (FBG) of all rats was determined before the start of the experiment. Blood sample was collected at weekly intervals from tail vein puncture till the end of study. In the continuous 21 days of drug treatment, a blood glucose level of all animals was determined at the 0, 7, 14, 21 day by using one touch glucometer (SD Check) method. On day 21, overnight fasted animals were under mild ether anaesthesia, the blood was collected by direct cardiac puncture. Blood was collected in tubes containing EDTA as anticoagulant for estimation of fasting plasma glucose and HbA1c.

Histological study

After blood sampling for the biochemical analysis, the animals were sacrificed, quickly dissected and small slices of pancreas were taken and fixed in 10% formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6µm in thickness were prepared and stained with Haematoxylin and Eosin then examined under microscopy [17].

Statistical study

All the values of body weight, fasting blood glucose level, and biochemical parameter estimations were expressed as mean ± standard error of mean (S.E.M) and was analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test, using InStat v.2.02 software (Graph Pad Software Inc.). Differences between groups (p Value) were considered significant at $P<0.05$ level. All data were graphically represented by using Prism Software V 2.02.

RESULT AND DISCUSSION

The preliminary phytochemical studies were done in the methanolic extract of *Daucus carota subsp sativus* leaves result that revealed of alkaloids, carbohydrate, flavonoids, phenolic compounds and tannins (Table 1).

Table 1: Phytochemical analysis of *Dacus carota subsp sativa* leaves extract

Test For Phytoconstituents	C.E	E.A.E	M.E
Saponins	+	-	+
Alkaloids	+	+	+
Glycosides	-	-	-
Tannins and phenolic compounds	+	+	+
Carbohydrates	+	+	+
Fixed oils	-	-	-
Flavanoids	+	+	+
Steroids	-	-	-

M.E-Methanolic extract, E.A.E-Ethyl acetate extract, C.E-Chloroform extract
+ Present, – Absent

Acute toxicity study

The acute oral toxicity of the methanolic extract of *Daucus carota subsp sativus* was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that LD50>2000mg/kg for the extract. Hence, the biological dose was fixed at MEDC 200mg and 400mg of body weight for the extract.

Effect On Glucose Tolerance

The doses of MEDC 200 mg/kg and 400 mg/kg increased the tolerance for glucose suggesting increased peripheral utilization of glucose. The reduction in blood glucose level was dose dependent (Table 2 and Figure 1).

Table 2: Effect of Glucose tolerance of methanol extract on streptozotocin induced diabetic rats.

Groups Treatment	Change in blood glucose levels (mg/dL)			
	Fasting	After 30 Minutes	After 60 minutes	After 90 minutes
Group 1: Normal Control (Vehicle only)	62.92±2.10	152.10±2.76	160.80±2.90	156.90±3.10
Group 2: MEDC 200mg/kg	68.76±1.5	130.08±3.87^{b}	146.52±3.26^{c}	141.76±3.18^{c}
Group 3: MEDC 400mg/kg	66.42±2.12^{a}	107.88±4.90^{a}	123.26±2.23^{a}	122.88±2.91^{a}
Group 4: Glibenclamide 3mg/kg	70.69±2.08	101.17±2.38^{a}	119.60±3.20^{a}	116.18±3.10^{a}

All values are expressed as mean ± SEM for six animals each statistically significant data*=$p<0.001^{b}=p<0.01;^{c}=p<0.05$.
MEDC treated groups (II,III) and standard group(IV) compared with control(I) group.

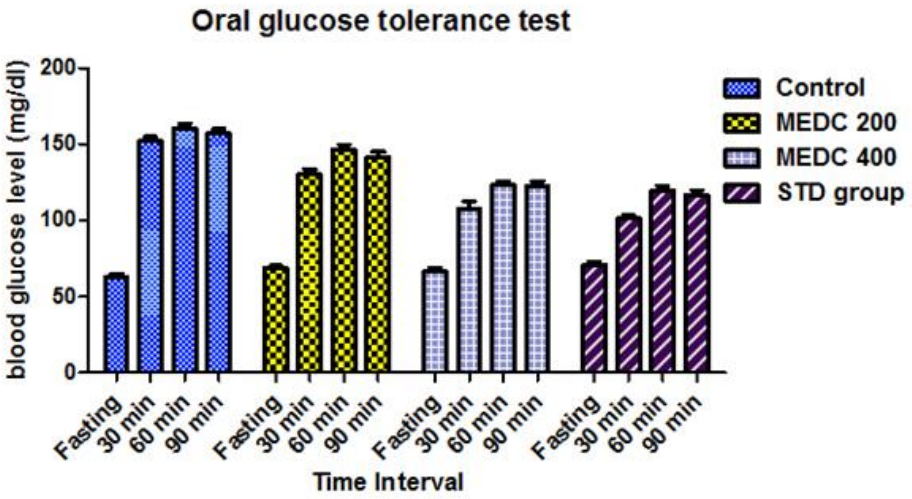


Figure 1: Effect of Glucose tolerance of methanol extract on streptozotocin induced diabetic rats.

Changes In Blood Glucose

A significant increase in the level of blood glucose was observed in diabetic control rats when compared to control rats. Administration of MEDC and glibenclamide to diabetic rats significantly decreased the elevated level of blood glucose, near to control level (Table 3 and Figure 2).

Table 3: Changes in Blood Glucose in methanol extract on streptozotocin induced diabetic rats.

Treatment	Blood glucose level (mg/dL)			
	Day 0	Day 7	Day 14	Day 21
Normal control rats (vehicles only)	78.60±3.14	79.10±3.12	82.96±2.56	76.84±2.91
Diabetic control rats	350.71±7.92[a]	366.18±12.96[a]	391.96±12.36[a]	397.10±11.64[a]
Diabetic group + Glibenclamide 3mg/kg	84.91±3.86[a]	81.76±4.36[b]	78.16±3.21a	66.67±1.22[a]
Diabetic group + MEDC (200mg/kg)	364.91±9.18[a]	324.21±5.90[a]	289.69±6.10[a]	260.71±9.90[a]
Diabetic group + MEDC (400mg/kg)	303.10±12.10[b]	266.90±0.20[a]	253.12±9.23[a]	220.86±11.92[a]

All values are expressed as mean ± SEM for six animals each statistically significantata*=p<0.001[b]=p<0.01;[c]=p<0.05.MEDC treated groups (II ,III) and standard group(IV) compared with control(I) group.

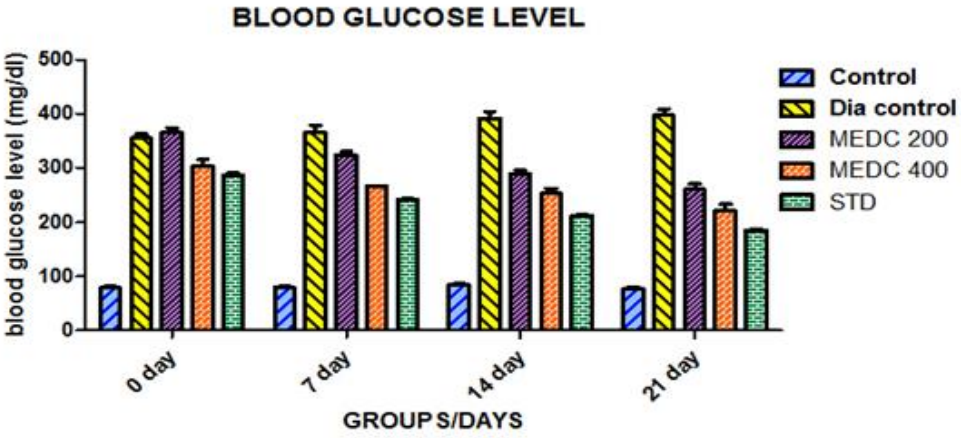
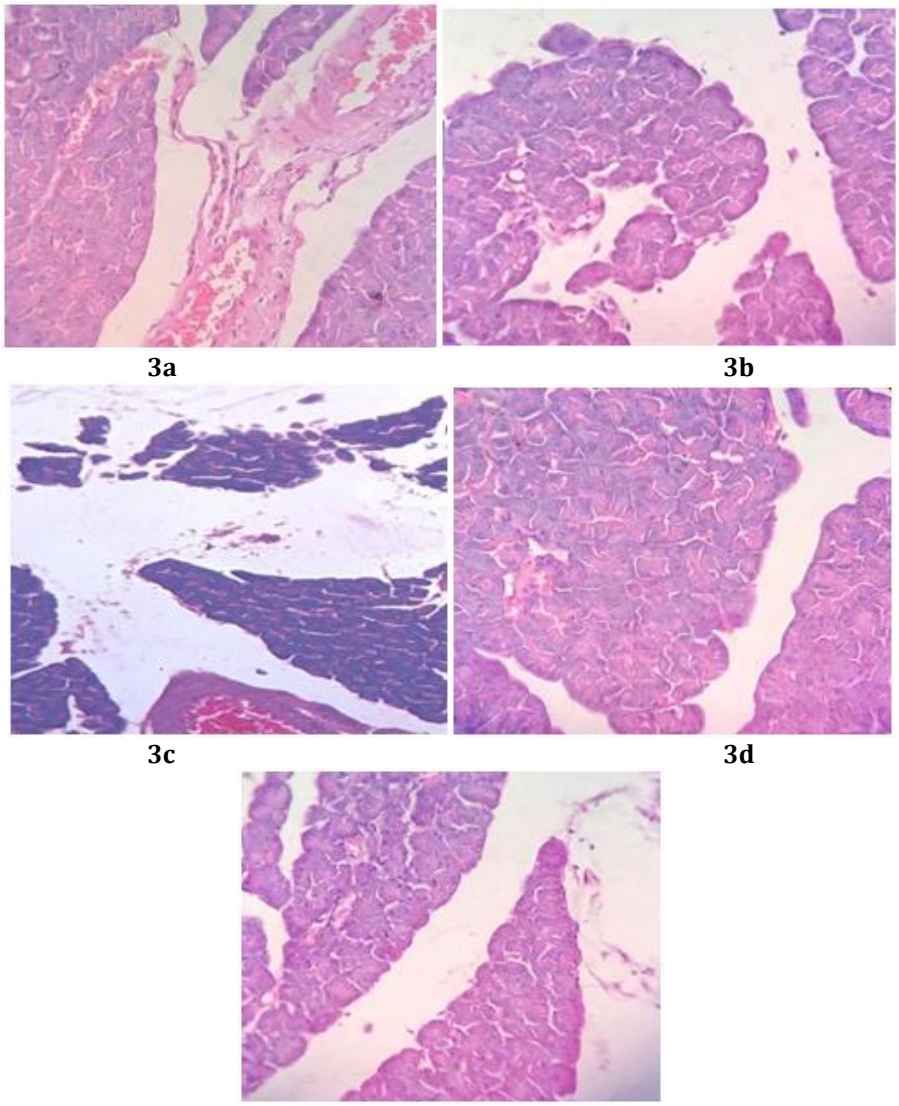


Figure 2: Changes in Blood Glucose in methanol extract on streptozotocin induced diabetic rats.

Histopathology Observation



3a **3b**

3c **3d**

3f

Figure 3: Histopathological slides of pancreas of different animal groups, Normal control (A); Diabetic control (B); Diabetic group with glibenclamide (C); Diabetic group with 200 mg MEDC (D); Diabetic group with 400 mg MEDC.

The anti-diabetic activity of methanolic extract was further confirmed by a histopathological study of the pancreas (Figure 3A-3D). Histology of the pancreas sections of the control rats showed the normal pancreatic β-cell. The pancreas sections of carbon streptozotocin treated rats showed the complete destruction of pancreatic β-cell due to the induction of streptozotocin when compared to normal control rats. The pancreatic sections of methanolic extract treated rats showed an increase in pancreatic β-cell count and remodelling of the structure of the pancreas when compared to the glibenclamide treated and control group's rats.

CONCLUSION

In the present study on the methanolic extract of *Daucus carota subsp sativus* leaves having antidiabetic activity more over nearest activity of glibenclamide. This study shows that flavonoids present in this extract may be possibly responsible for the antidiabetic activities. Histopathological studies on isolated pancreas revealed that methanolic extract of *Daucus carota subs psativus* reversed the changes which produced due to diabetes caused by streptozotocin. The normal pattern of histology of pancreas was observed and further pharmacological and biochemical investigation is to be done to find out the active constituent responsible for the antidiabetic activity.

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