# EVALUATION OF ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF *ARTEMISIA DRACUNCULUS* EXTRACTS IN STREPTOZOTOCIN-INDUCED-DIABETIC RATS

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**Summary**

The aerial and root extracts of *Artemisia dracunculus* were subjected to screening of antidiabetic activity in Streptozotocin (STZ)-induced diabetic rats. The ethanol and aqueous extracts (250 mg/kg body weight) were administered orally in diabetic rats. After the oral administration of ethanol and aqueous extracts of aerial and root of *Artemisia dracunculus*, blood glucose levels were monitored at specific intervals and it was found that they were significant lowered. In the same study the action of the extracts on diabetes induced hyperlipidemia was analyzed where the extracts significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the High density lipoprotein (HDL). Glibenclamide was used as a standard drug at a dose of 0.25 mg/kg. The experimental data revealed that extracts of aerial and root of *Artemisia dracunculus* has significant antihyperglycemic activity in Streptozotocin-induced rats compared to the standard drug. The drug has the potential to act as antidiabetic as well as antihyperlipidemic.

**Keywords** *Artemisia dracunculus*, antidiabetic, glibenclamide, streptozotocin

## Introduction

Diabetes mellitus is a metabolic disorder due to relative or absolute lack of insulin or its improper utilization, resulting in elevated blood glucose levels in association with long term vascular and neurological complications. The diabetes mellitus may be insulin dependent diabetes mellitus or non-insulin dependent diabetes mellitus depending upon the conditions of insulin requirement in the body. The prevalence of insulin dependent diabetes mellitus (IDDM) is 10% whereas that of non-insulin dependent diabetes mellitus (NIDDM) is 90% of the diabetic population. Diabetes mellitus, the most pervasive and costly chronic disease is afflicted by an estimated 175 million people worldwide. It is a leading cause of adult blindness and end-stage kidneys disease. Additionally diabetics are two to four times more likely to have heart disease or to suffer a stroke. Therefore, Diabetes mellitus has been recognized as a growing world-wide epidemic by many health advocacy groups including the World Health Organization (WHO).

Every year the number of diabetic patients is growing alarmingly all over the World. Most of the hypoglycemic agents used in allopathic medicines are reported to have side effects in the long run[1,2]. Therefore, there is a need to search for effective and safe drugs for diabetes.

*Artemisia dracunculus* (Asteraceae) popularly known as Tarragon, perennial green, erect herb, found in the alpine regions of Kashmir and distributed in the western Himalayas at altitudes of 4,200-4,800 m. stems 30-60 cm in height, grooved and ribbed; leaves 2.5-3.6 cm long, linear or linear-oblong, entire; flower heads almost round 3mm in diameter, sessile or stalked in branching racemes, sometimes clustered in threes, bracts of heads broadly oblong with a very broad transparent margin and a very narrow green disc. The leaves are popular in French cooking, usually in mild-flavored dishes, such as chicken and fish dishes, eggs, sauces, salads and pickles. Tarragon is indispensable in sauce Bearnaise, and makes a fine flavouring for vinegar or mustard. In traditional folk medicine, tarragon has been used for digestive problems and intestinal worms, and externally for joint pain. It is mentioned in the PDR for Herbal Medicines but is not classified as an approved herb by the German Commission E, and some sources do not recommend it for pregnant women. Tarragon is also used as a commercial flavouring and in perfumery. In India *A. dracunculus* is commonly used in the treatment of various diseases like antimalarial, neuromascular antispasmodic, anti inflammatory, carminative, diabetes and appetite, anticonvulsant activity, hyperglycemia, antifungal[3-9]. The core objectives of this study was to assess the antidiabetic potential of ethanol and aqueous extracts of the powdered aerial and root of *Artemisia dracunculus* in control of blood glucose levels and effectiveness on various biochemical parameters, namely, total cholesterol, triglycerides (TGL), high density lipoprotein (HDL) and low density lipoprotein, (LDL).

## Material and Methods

**Plant materials:** The aerial and root part of *Artemisia dracunculus* were collected from the forest area of Leh region, Jammu and Kashmir, India, during the months of February 2011. The species was identified by the local people during the time of collection and later on authentication was made by botanist Dr. S. N. Sharma Department of Taxonomy, I.I.I.M, Jammu, India and a voucher specimen was deposited in the Herbarium of Department of Botany, IIIM Jammu. The aerial and root were shade dried, reduced to coarse powder and stored in airtight container till further use.

**Preparation of extract:** 1 Kilogram of aerial and root powdered drug were packed in soxhlet apparatus separately and extracted with ethanol. The ethanol extract of both parts were separated and the marcs were further extracted with distilled water. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The ethanol extract of aerial and root were abbreviated as BDAB/080/A/A001 and BDAB/080/R/A001, while aqueous extracts of aerial and root were abbreviated as BDAB/080/A/A002 and BDAB/080/R/A002.

**Experimental animals:** Male wistar albino rats having weight 180-230 gm bred in the Animal House, Institute of Integrative Medicine (IIIM), Formerly Regional Research Laboratory (CSIR), Jammu, were used. The animals were housed were kept in quarantine for 10 days under standard husbandry conditions. All animal used in experiments were approved by the Institutional Animal Ethic Committee (IAEC), of Indian Institute of Integrative Medicine, IIIM (Formerly Regional Research Laboratory) (CSIR) Jammu. 3$^{o}$C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad. libitum*.

**Acute oral toxicity study:** Acute oral toxicity was performed by following OECD guideline – 420 fixed dose procedure for ethanol and aqueous extract and it was found that dose increasing up to 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The $LD_{50}$ of the ethanol and aqueous extract as per OECD guidelines – 420 is greater then 2000 mg/kg[9,10].

**Oral glucose tolerance test (OGTT):** The oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. The rats were divided into seven groups ($n$ = 6) and were administered drinking water. Group I served as normal control rats administered drinking water; Group II glucose control rats administered; Group III rats administered standard drug glibenclamide (0.25 mg/kg); Group IV diabetic rats administered BDAB/080/A/A001 (250 mg/kg); Group V diabetic rats administered BDAB/080/A/A002 (250 mg/kg); Group VI diabetic rats administered BDAB/080/R/A001 (250 mg/kg); Group VII diabetic rats administered for BDAB/080/R/A002 (250 mg/kg) 28 days. Glucose (2 g/kg) was fed 30 minutes prior to the administration of the extracts. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 minutes of extract administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit[11].

**Induction of non-insulin dependent diabetes mellitus (NIDDM):** Non-insulin dependent diabetes mellitus was induced in overnight fasted adult Wistar strain albino male rats weighing 180–230 gm by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 126 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study[12,13].

**Experimental design:** The animals were segregated into seven groups of six rats each. The extract was administered for 28 days. Group I served as normal control rats administered drinking water daily for 28 days; Group II diabetic control rats administered drinking water daily for 28 days; Group III diabetic rats administered standard drug glibenclamide (0.25 mg/kg); Group IV diabetic rats administered BDAB/080/A/A001 (250 mg/kg); Group V diabetic rats administered BDAB/080/A/A002 (250 mg/kg); Group VI diabetic rats administered BDAB/080/R/A001 (250 mg/kg); Group VII diabetic rats administered for BDAB/080/R/A002 (250 mg/kg) 28 days.

The fasting glucose levels were determined on days 0, 7, 14 and 28 of extract administration. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated.

**Estimation of biochemical parameters:** The biochemical parameters were determined on day 12 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined by the glucose oxidase method, using an auto-analyzer[14-16].

**Statistical analysis:** Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Sigma stat 3.5). P values <0.05, <0.01, <0.001 were considered statistically significant.

## Results

The result of acute toxicity study of ethanol and aqueous extracts of *Artemisia dracunculus* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. The effects of ethanol and aqueous extracts of both parts of *A. dracunculus*, on the plasma glucose level are shown in table 1. Both the extracts treated rats as well as standard drug, significant reduction in plasma glucose level, while in glucose control rats, plasma glucose level was increased.

Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of both extract of aerial and root of *A. dracunculus*, on fasting plasma glucose level of normal and streptozotocin induced are shown in table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels were statistically significant in diabetic rats.

The effect of the both extracts of aerial and root on diabetes induced hyperlipidemia was also studied. It was observed that due to diabetes there was an increase in the total cholesterol levels as well as triglyceride levels. The HDL levels were reduced in the diabetic animals and the LDL levels were increased significantly (Table 3).

Both the extracts of aerial and root showed a significant decrease in the total cholesterol levels and triglyceride levels. In particular, the ethanol extract of aerial of 250 mg/kg body weight showed a much relevant action. It also increased the HDL level and was successful in suppressing the LDL levels as compared to the standard drug (Table 3).

## Discussion

The result of acute toxicity study of ethanol and aqueous extracts of *A. dracunculus* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed as per OECD guidelines both the

extracts fall under class four values $LD_{50}$ value being 2000 mg/kg. The pharmacological evaluations were therefore carried out at doses of 100 and 200-mg/kg body weight.

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production and decreased utilization of glucose by the tissues. In our study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 12-day experimental period. When ethanol and aqueous extracts of aerial and root of *A. dracunculus* were administered to glucose loaded normal rats fasted for 18 h, decrease in plasma glucose level was observed after 30 min. Both the extracts reduced plasma glucose level to normal at 90 min. During study it was found that both extracts control significantly the blood glucose level on streptozotocin induced diabetic rats. The ethanol and aqueous extracts of aerial and root induced a significant reduction on blood glucose level in STZ-induced-diabetic rats as compared to the diabetic control group. But ethanol extract of aerial and root parts showed more significant antidiabetic activity as compared to aqueous extract of aerial and root. The possible mechanism by which *Artemisia dracunculus* brings about its hypoglycemic action in diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

The marked increase in serum triglycerides and cholesterol observed in untreated diabetic rats. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the both extracts of aerial and root treated diabetic rats may be directly attributed to improvements in insulin levels upon *Artemisia dracunculus* therapy. Elevation of plasma lipid concentration in diabetes is well documented. In insulin deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis. Induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes. Diabetic rats treated with the extracts showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis[17].

Abnormalities in lipoproteins are very common in both NIDDM and IDDM. Although lipoprotein alterations appear to be an intrinsic part of these disorders, such alterations are also induced by diabetes associated complications such as obesity and renal disease. The total cholesterol, triglyceride levels, LDL and LDL were observed to be elevated in diabetics but reduced by both extracts as well as glibenclamide showing their beneficial effects. In the present study, HDL levels remained unchanged in diabetics compared to the other groups. These results suggest the beneficial effects of the natural extract in improving the imbalance in lipoprotein metabolism are also comparable to those of glibenclamide.

The present study has indicated the fact that the plant *Artemisia dracunculus*, has antidiabetic and antihyperlipidemic constituents and production of a safe antidiabetic drug is very much possible from the aerial and root part.

**Table 1: Effect of ethanol and aqueous extracts of *A. dracunculus* on oral glucose tolerance test**

| Group | Blood Glucose level (mg/dl) | | |
|---|---|---|---|
| | 0 min | 30 min | 90 min |
| Normal Control | 75±3.7 | 69±2.9 | 72±1.0 |
| Glucose control | 84±3.3 | 174±6.4 | 142±4.3 |
| Glucose + Glibenclamide | 91±3.9 | 132±3.2*** | 87±3.8*** |
| BDAB/080/A/A001 | 94±2.2 | 165.8±6.77 | 133±4.1** |
| BDAB/080/A/A002 | 91±3.9 | 171±6.5 | 149±4.6** |
| BDAB/080/R/A001 | 89±3.1 | 148±8.2*** | 103±5.5*** |
| BDAB/080/R/A002 | 93±4.8 | 169±7.5* | 146±6.6** |

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with diabetic control group.

**Table 2: Effect of ethanol and aqueous extracts of *A. dracunculus* fasting plasma glucose level in rats**

| Group | Fasting plasma glucose concentration (mg/dl) | | | |
|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day 28 |
| Normal control | 96±4.98 | 92.6±5.4 | 100.3±18.6 | 87±4.0 |
| Diabetic control | 106±3.84 | 259.2±14.3 | 235.7±30.3 | 215.3±12.5 |
| Diabetic + Glibenclamide | 95±9.42 | 284.2±9.4 | 151±21.3* | 117.8±13.9** |
| Diabetic+ BDAB/080/A/A001 | 94±3.54 | 279.5±3.7 | 185.3±23.3 | 126.2±6.7*** |
| Diabetic+ BDAB/080/A/A002 | 99±4.01 | 274±32.4 | 195.7±14.0 | 149±6.1** |
| Diabetic+ BDAB/080/R/A001 | 81±4.52 | 251±12.6 | 175.3±10.9 | 155.5±5.8*** |
| Diabetic+ BDAB/080/R/A002 | 81±8.45 | 260±12.7 | 215.8±14.7 | 175.6±5.5* |

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with diabetic control group.

**Table 3: Determination of biochemical parameters after treatment with ethanol and aqueous extracts of *A. dracunculus* and Glibenclamide**

| Group | Lipid Profile (mg/dl) | | | |
|---|---|---|---|---|
| | Triglyceride | Total Cholesterol | HDL | LDL |
| Normal control | 83.3±2.7 | 98±1.2 | 54.5±2.1 | 44±3.2 |
| Diabetic control | 110.7±1.3 | 146.7±4.4 | 33.6±1.7 | 113.3±1.8 |
| Diabetic + Glibenclamide | 84±1.0*** | 107.7±1.6*** | 56±1.6** | 51.6±2.2 |
| Diabetic+ BDAB/080/A/A001 | 90±1.2** | 113.3±2.2** | 53.5±1.5** | 58.2±2.2 |
| Diabetic+ BDAB/080/A/A002 | 91.1±1.4*** | 126±1.3** | 52.3±2.1** | 74.2±1.3 |
| Diabetic+ BDAB/080/R/A001 | 96.1±2.7** | 119.8±1.6*** | 51±1.2** | 64.7±3.6 |
| Diabetic+ BDAB/080/R/A002 | 101.7±0.7** | 133.3±1.7* | 45.8±2.3 | 84.7±2.8 |

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with diabetic control group.

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