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# Phytochemical screening and hypoglycemic effect of *Artemisia dracunculus* L.

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ABSTRACT

**Background:** Diabetes is a metabolic disorder associated with hyperglycemia and caused by defect in insulin secretion. The aim of the present study was to evaluate the antidiabetic activity of *Artemisia dracunculus L* in alloxan induced diabetic rats.

**Material and methods:** The antidiabetic effect of *Artemisia dracunculus L* was studied against alloxan (140 mg/k g b.w., i.p.) induced diabetes in Wistar rats for doses 250 mg/kg b.w. and 500 mg/kg b.w. (p.o.) for four weeks and the effect was compared with standard glibenclamide (10 mg/kg, b.w).

**Results:** Diabetes induced by alloxan treatment increases the level of glucose and biochemical parameter in blood sample but treatment with *Artemisia dracunculus* L significant decrease the elevated glucose and blood biochemical parameter.

**Conclusion:** Hence, the results obtained in the present study indicate that *Artemisia dracunculus* L posses significant anti-diabetes activity.

**Keywords:** *Artemisia dracunculus* L, Alloxan, Biochemical parameter, Glibenclamide.



## INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4% in 2025. It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many parts of the body system, particularly the blood vessels and nerves (Kim et al., 2006). Several botanical neutraceuticals/supplements have been studied as potential therapeutic agents in the management of diabetes and its related complications.

Tarragon (*Artemisia dracunculus* L.) of the family Asteraceae is a perennial herb with a long history of medical and culinary use. There are two main cultivars of *A. dracunculus* L. known as French tarragon (*Artemisia dracunculus* var. sativa) and Russian tarragon (*Artemisia dracunculus* var. inodora (Vienne et al.,1989). French tarragon is a sterile plant, reaching a height of approximately 70 cm. It is sensitive to low temperature and its aromatic flavor can be described as spicy, slightly burning and anise-like. Russian tarragon with a growth of approximately 1.5m is fertile. It is frost tolerant and reveals a weak aroma. Its flavor is slightly bitter and chervil-like (Haeusel et al., 1992; Teuscher, 2003). The drug from *Artemisia dracunculus* L. is Dracunculi herba. Polyphenols such as 6-demethoxycapillarisin, 2′, 4′-dihydroxy-4-methoxy-dihydochalcone and 4, 5-di-O-caffeoylquinic acid, flavonoids such as luteolin and apigenin, the coumarin scopoletin and the sesquiterpenoid lactone costunolide as well as cinnamates represent the main components in Dracunculi herba (Logendra et al., 2006 and Govorko et al., 2007). In 1989 Vienne et al. demonstrated that the flavonole profile of Russian tarragon clearly differs from that of French tarragon in the amount of quercetin and especially of patuletin glycosides, which seem to occur only in the Russian cultivar.

While *A. dracunculus* is used in traditional medicine mainly as a remedy for gastrointestinal disorders, recent studies reported possible antidiabetic and glucose-lowering effects (Ribnicky et al., 2009 and Wang et al., 2008). *A. dracunculus* was also tested for its potential beneficial effects on the metabolic syndrome (Cefalu et al., 2008). It was demonstrated that an ethanol extract of *A. dracunculus* was able to reduce blood glucose concentrations in rodents with chemically induced

diabetes, as well as in genetically diabetic rodents with insulin resistance (Ribnicky et al., 2006).

## MATERIALS AND METHOD

### Preparation of plant extract

100 g of *Artemisia dracunculus* leaves was powdered, dried and continuously extracted for 48hrs with hydroalcoholic in a Soxhlet apparatus. The collected extract was stored at 0-4°C until used. The plant extract was pooled and evaporated to dry at 60 °C.

### Preliminary phytochemical screening

Preliminary phytochemical investigation was carried out on hydroalcoholic extract of *Artemisia dracunculus* for detection of various phytochemicals by following standard methods described in practical Pharmacognosy by CK Kokate and RK Khandelwal.

### Acute toxicity

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD) 423 guidelines. Three male Wistar rats weighing between 150-200 g were used for each dose. The dose levels of 5mg, 50 mg, 500 mg, 1000 mg, 2000 mg and 3000 mg/kg/body weight, were selected. The lethal dose LD-50 value of the extract was determined. The drug was administered orally to rats, which fasted overnight with water ad libitum before the administration of the drug. The body weight of the rat was noted before and after treatment. The animals were observed for toxic symptoms, behavioural changes, locomotion, convulsions and mortality for 72hrs.

### Experimental animals: alloxan induced diabetic model:

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilised with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

### Experiment design

Total of 30 rats were divided in to 5 groups (n=6) as follows:
Group I: Served as normal control and did not receive any treatment.
Group II: Served as diabetic control and received alloxan monohydrate and vehicle.
Group III: Alloxan (150 mg/kg)+Glibenclamide (10 mg/kg p.o.) served as standard.
Group IV: Alloxan (150 mg/kg)+hydroalcoholic leaves extract of *Artemisia dracunculus* (250 mg/kg, p.o.)
Group V: Alloxan (150 mg/kg)+hydroalcoholic leaves extract of *Artemisia dracunculus* (500mg/kg, p.o.)

### Treatment schedule

Group-I nondiabetic animals: received only 1% gum acacia (1 ml/kg/day, p.o.) for four weeks, and served as control. Group-II to V was rendered diabetic by single intraperitoneal dose of alloxan monohyderate 150 mg/kg, in citrate buffer (pH 4.5). Group II received 1 % gum acacia (1 ml/kg/day, p.o.) for four weeks and served as diabetic control. Group-III received glibenclamide (10 mg/kg/day, p.o.) for four weeks. Group-IV and V received two different doses of *Artemisia dracunculus* (250 and 500 mg/kg/day, p.o.) for four weeks respectively.

**Care of diabetic animals:** Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and in some circumstances, more than once per day. Diabetic rats should have sufficient food and water.

**Collection blood and serum samples:** The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasion, i.e., 0, 10th, 20th and 30th day. The blood samples were allowed to clot for 30mins at room temperature and then they were centrifuged at 3000 rpm for 10 mins. The resulting upper serum layer was collected in properly labelled, clean and dry micro-centrifuge tubes. The serum samples were stored at -400 °C and analyzed either immediately or within two weeks. The parameters studied were as follows: Biochemical parameters such as

a. Serum total cholesterol.
b. Serum and creatinine.
c. Serum urea.
d. Serum total protein.

- Body weight of an animal.
- Blood glucose

### Statistical analysis

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

## RESULTS

### Preliminary phytochemical screening

Results of the preliminary phytochemical investigation of hydroalcoholic leaves extract of *Artemisia dracunculus* are shows the presence of Alkaloids, Carbohydrates, Steroids, Protein, Tannins, Phenols, Flavonoids, Glycosides etc.

No acute toxicity was observed for hydroalcoholic leaves extract of *Artemisia dracunculus* when it was administered orally at high dose level (3 g/kg body weight), which is higher than effective antihyperglycemic dose, and closely observed for 24 hrs

for any mortality and next 10 days for any delayed toxic effects on gross behavioural activities.

**Hypoglycemic study of alloxan induced diabetic rats**

**a) Body weight**

The diabetic control showed significant decrease in the body weight during the treatment period. The diabetic animals treated with *Artemisia dracunculus* (250 mg/kg) showed slight reduction in body weight but not much when compared to control. The group that received *Artemisia dracunculus* 500 mg/kg had shown significant results (Table 1).

**Table 1. Effect of *Artemisia dracunculus* on body weight in alloxan induced diabetic rats**

| Groups | Body weight of the animal (g) | | | |
|---|---|---|---|---|
| | Initial | 10th day | 20th day | 30th day |
| Normal | 153±1.062 | 161±3.01 | 170±2.56 | 180±4.65 |
| Alloxan + Vehicle | 174±2.14 | 163±3.20 | 153±2.30 | 140±2.28 |
| Alloxan + Glibenclamide 10 mg/kg | 169±2.22** | 162±2.53** | 172±4.2** | 181±2.64*** |
| Alloxan + *Artemisia dracunculus* 250 mg/kg | 159±2.20* | 157±1.32* | 161±2.80* | 164±2.24* |
| Alloxan + *Artemisia dracunculus* 500 mg/kg | 160±3.22** | 158±5.24** | 165±8.22** | 172±3.28*** |

Values are Mean ±SEM; n=5

ns= non-significant, *P <0.05, **P < 0.01 and ***P<0.001 vs. Diabetic Control.

**b) Blood glucose**

The Diabetic control showed significant increase in the blood glucose during the treatment period. The diabetic animals treated with *Artemisia dracunculus*(250mg/kg) showed slight reduction in blood glucose but not much when compared to control. The group that received *Artemisia dracunculus* 500mg/kg had shown significant results (Table 2).

**Table 2. Effect of different groups on blood glucose (mg/dl) level in alloxan-induced diabetic.**

| Groups | Blood glucose level (mg/dl) | | |
|---|---|---|---|
| | Day 1 | Day 15 | Day 30 |
| Normal | 66.5± 3.1 | 67.1± 4.3 | 66.5± 2.6 |
| Alloxan+vehicle | 238.4± 2.6 | 278.2± 4.2 | 310.6± 3.4 |
| Alloxan+Glibenclamide (10 mg/kg) | 242.6± 6.8 | 110.4± 6.0** | 68.8± 8.2** |
| Alloxan+ *Artemisia dracunculus* (250 mg/kg) | 252.2± 6.2 | 130.4± 4.2** | 90.2± 5.4** |
| Alloxan+ *Artemisia dracunculus* (500mg/kg) | 246.2± 1.1 | 118.6± 2.6** | 72.4± 4.6** |

Values are mean ± SD n=6 in each group

**p<0.01 as compare to control group.

**c) Biochemical parameters**

Diabetic animals treated with *Artemisia dracunculus* showed significant decrease in serum creatinine, serum cholesterol and urea, and significant increase in serum total protein when compared with diabetic control (Table 3).

**Table 3. Effect of *Artemisia dracunculus* on biochemical parameters in alloxan-induced diabetic rats.**

| Groups | Serum protein (mg/dl) | Serum urea (mg/dl) | Serum create-nine | Serum choles terol |
|---|---|---|---|---|
| Normal | 6.9± 0.8 | 38.4± 0.4 | 0.74± 0.02 | 73.20± 43.6 |
| Alloxan+ Vehicle | 4.3±0.4 | 84.8± 2.4 | 1.82± 0.04 | 134.52 ±6.4 |
| Alloxan+ Glibenclamide (10 mg/kg) | 6.8± 0.1** | 40.1± 2.4** | 0.76± 0.02*** | 80.64± 2.4*** |
| Alloxan+ *Artemisia dracunculus* (250 mg/kg) | 5.6± 6.8* | 72.2± 6.4* | 1.40± 0.06* | 99.42± 6.8* |
| Alloxan+ *Artemisia dracunculus* (500 mg/kg) | 6.2± 0.2** | 42.6± 2.5** | 0.80± 0.08*** | 75.64± 5.2*** |

Values are Mean ± S.E.M; n=6

* P<0.05, **P < 0.01 and ***P < 0.001 vs. Diabetic Control

## DISCUSSION

Herbal drugs are normally used as compound preparations called decoctions, powders and pastes to treat the patients with Type 2 diabetes in Ayurveda and traditional systems of medicine(May et al.,2002). Treatment of hyperglycemia in diabetes involves diet control exercise and the use of hypoglycemic diets and drugs. However, many oral antidiabetic medicines have a number of serious adverse effects.

Human diabetics and experimental diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which may result in depletion of the antioxidant defense system and lead to an enhanced de novo free radical generation (Kamalakannan et al., 2006). In addition, high glucose contents can simply

inactivate antioxidant enzymes (Davi et al., 2005). Reaction of these free radicals with membrane lipids would result in an increased lipid peroxidation which can be prevented by antioxidants including plant phenolic compounds (Coskun et al., 2005).

Diabetes mellitus ranks highly among the top ten disorders which cause mortality throughout the world. Diabetes mellitus being chronic disorder, treatment without side effect for long term control is important. Present antidiabetic agent possess side effect as risk of hypoglycemia, anemia, cholestatic jaundice (Schimmer et al., 2001). There has been growing public interest in herbal medication for treatment of diabetes.

In the present study the periodic estimation of plasma glucose revealed that *Artemisia dracunculus* produced significant antihyperglycemic activity which began from 22nd day of treatment and it progressed throughout the study. The antidiabetic effect of the *Artemisia dracunculus could* possibly be due to presence of glycosides, tannins and saponins. Substances like glycosides, alkaloids, terpenoids and tannins are frequently implicated as having antidiabetic effects (Matsuda et al., 2002). However, since it has been shown previously that an ethanol extract of *Artemisia dracunculus* L, showed antihyperglycemic activities (Ribnicky et al., 2006).

The bioactivity guided fractionation of the extract, using *in vitro* assays related to diabetes, lead to the identification of 6 active compounds that may contribute to the hypoglycemic activity observed *in vivo* (Schmidt et al., 2008a). The identification of these compounds and their activities reinforce the multi component and multi mechanistic advantage that botanical preparations may provide over single chemical entities (Raskin et al., 2002). Their potential role as active compounds *in vivo*, however, is only indirectly supported by *in vitro* results and can only be validated with *in vivo* studies using the combinations of purified compounds.

Various reports suggest that there is reduction in the body weight in diabetic rats. Loss of body weight could be due to, dehydration and catabolism of fats and protein seen during diabetes mellitus (Hofteizer et al., 1973). It is reported that the recovery in body weight is far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. In the present study diabetic control group rats showed significant loss of body weight. All animals treated with *Artemisia dracunculus* showed significant prevention of the loss in body weight throughout the study. This prevention of loss in body weight by *Artemisia dracunculus* may be due to increasing glucose uptake in peripheral tissues or inhibiting catabolism of fat and protein or by glycemic control.

Diabetes produces qualitative and quantitative changes in the composition of the basement membrane and this altered material undergoes accelerated glycosylation and further rearrangement to form advanced glycation end-products (AGEs), which stimulate protein synthesis, further decrease degradability of the basement membrane, increase its permeability and cause endothelial dysfunction. Hyperglycemia increases the expression of transforming growth factor beta (TGFß) in the glomeruli and of matrix protein specifically stimulated by cytokine. TGFß may contribute to both the cellular hypertrophy and enhanced collagen synthesis is observed in diabetic nephropathy (Vishwanathan et al., 2004).

During diabetes, there is increased protein catabolism with inflow of amino acids to liver, which feed gluconeogenesis and accelerate ureagenesis, resulting in hypoproteinemia and hypoalbuminemia (Bhavpriya et al., 2002). Diabetic hyperglycemia induces elevation of the levels of serum creatinine, urine total protein and urine albumin which are considered as significant markers of renal dysfunction (Bretzel et al., 1997).

In the present study, diabetic animals treated hydroalcoholic leaves extract of *Artemisia dracunculus* showed reduction in body weight, glucose levels and also showed improvement in the serum total protein level. Treatment with *Artemisia dracunculus* also prevented the rise in serum creatinine levels. These results indicate that *Artemisia dracunculus* attenuates the progression of renal damage in alloxan induced diabetic rats. The use of typical antioxidants alone or in combination may retard or even prevent the normal progression of diabetic complications (Sabu et al., 2002). Hence, the results obtained in the present study indicate that hydroalcoholic leaves extract of *Artemisia dracunculus* has the potential to treat diabetes mellitus.

## CONCLUSION

In the present study the hydroalcoholic leaves extract of *Artemisia dracunculus* shown better hypoglycemic activity in experimental rat models, it may be due to the presence of flavonoids and other poly phenolic compounds. Hence, the research justifies that the hydroalcoholic extract of *Artemisia dracunculus* leaves can be effectively used in treatment of diabetes by reducing the body weight and glucose levels. Further studies are needed to isolate and characterize the active component(s) responsible for the anti-diabetic properties of the test extract and findings should be confirmed by performing clinical studies.

## CONFLICT OF INTEREST

None declared.

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