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# Hypoglycaemic Activity of Flower Heads of *Artemisia Maritima* in Normal and Alloxan-induced Diabetic Rats

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## Abstract

Anti-diabetic activity of flower heads of *Artemisia maritima* in normal glucose-loaded and alloxan-induced diabetic rats was studied. Oral administration of the alcoholic extract of flower heads of *Artemisia maritima* extract was given daily at a dose of 250 and 500 mg/kg body weight to alloxan-induced diabetic rats for a period of 14 days. Significant reduction in blood glucose level in alloxan-induced diabetic rats was noticed. The study indicates that alcoholic extract of flower heads of *Artemisia maritima* at a dose of 250 and 500 mg/kg body weight showed statistically significant reduction, but 500 mg/kg body weight showed more statistical significant effect as compared to the dose at 250 mg/kg body weight.

**Keywords:** Alloxan, diabetes, *Artemisia maritima*, blood glucose

## 1. Introduction

Diabetes mellitus is a chronic disease characterised by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels [1]. Diabetes mellitus is a worldwide health problem afflicting millions in both developed and developing countries. It is the primary cause of chronic kidney failure, blindness, high blood pressure and premature coronary artery disease. Diabetes mellitus was known to ancient Indian physicians as 'madumeha'. Many herbal products including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones [2]. Many herbs have been shown to have hypoglycemic action in animals and humans [3]. In recent years, emphasis has been on the development of drugs from plants for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world, especially in India [4]. Alloxan diabetic model resembles type 1 diabetes (insulin dependent diabetes mellitus) without significant insulin resistance [5].

*Artemisia maritime,* commonly known as Santonica, is a small deciduous perennial shrub with much-branched woody rootstalk, up to 100 cm in height belonging to family Asteraceae [6]. Flower heads, when rubbed, have a strong agreeable odour [7]. Flowers are yellow in colour while all other parts are whitish grey and are bitter and camphoraceous in taste [8]. It is a bitter aromatic herb or low shrub with much-divided leaves and inconspicuous flowers born on numerous small heads [9]. The plant is reported to possess hepatoprotective [10], insecticidal [11], antimicrobial, anthelmintic, cytotoxic and anti-tumor activity [12]. Traditionally, fresh and dried extracts of top foliage and shoots are given to children against infestation of tapeworms and roundworms. It has been found that no

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specific work has been done on alloxan-induced diabetic rats using flower heads of *Artemisia maritima*. So it was considered worthwhile to investigate the *Artemisia maritima* alcoholic extract in normal glucose-loaded and alloxan-induced diabetic rats.

## 2. Materials and Methods

### 2.1 Plant Material

The flower heads of *Artemisia maritima* were collected from Budgam district of Kashmir valley in the month of July. The plant was identified and authenticated by Botanist Dr. Saroj Arora, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. Its voucher (0401/Hbr) specimen was deposited in the same department for reference.

### 2.2 Preparation of Alcoholic Extract

The ethanolic extract (95%) of the plant was prepared by extracting successively the flower heads (500 gm) with 2 l of alcohol in a Soxhlet extractor for 36 hr. Then the liquid extract was concentrated on vacuum rotary evaporator to yield dry residue of 30 g (7.5% yield) and was stored at 4 °C. The hypoglycaemic effect was evaluated by oral administration of the extract on normal glucose-loaded and alloxan-induced diabetic rats.

### 2.3 Animals

Adult albino rats of either sex, weighing about 100–150 g, were used in the present investigation. Male and female animals were housed separately in groups of six per cage (polycarbonate cage size: 29×22×14 cm) under laboratory conditions with alternating light and dark cycle of 12 hr each. The animals had free access to food and water. All the rats were given a period of acclimatisation of 15 days before starting the experiment. They were fed ad libitum every day with standard chow diet (Hindustan Lever, India) and were given free access to water. Animals described as fasting were deprived of food for at least 16 hr but were allowed free access to drinking water. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC), and animal care was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No. SBS/3235).

## 3. Effect of Alcoholic Extract of *Artemisia Maritima* Linn. on Normal Glucose-Loaded Rats

### 3.1 Effect of Test Drug on Oral Glucose Tolerance (OGTT)

Fasted normal rats were divided into five groups of six animals each. Group 1 served as control and received vehicle only. Group 2 received the reference drug glimepride at an oral dose of 150 mg/kg body weight. Group 3 received ethanol extract of *Artemisia maritima* at an oral dose of 250 mg/kg body weight. Group 4 received ethanol extract of *Artemisia maritima* at an oral dose of 500 mg/kg body weight. Group 5 received insulin in the form of injection through i.p. route at a dose of 3 I.U/kg. After 30 min of extract/drug administration, the rats of all the groups were orally treated with 2 g/kg body weight of glucose. Blood samples (0.2 ml each sample) were collected from the tip of the tail just prior to glucose administration and at 30 and 90 min after glucose loading. Serum was separated, and blood glucose levels were measured immediately by glucose oxidase method [13].

## 4. Induction of Experimental Diabetes

Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate (S.D Fine-Chem. Ltd., Mumbai, India) in sterile saline [14]. After 72 hr of alloxan injection, the diabetic rats were separated and used for the study.

Alloxan monohydrate was dissolved in sterile normal saline immediately before the use and was injected intraperitoneally to 18–hr-fasted rats at a dose of 120 mg/kg/ body weight. After alloxanisation, the animals were given feed *ad libitum* and 5% dextrose solution for the next 24 hrs to overcome initial hypoglycaemic phase due to massive pancreatic insulin release caused by alloxan. The blood glucose levels (BGL) were monitored after alloxanisation in blood samples collected by tail tipping

method. The blood was dropped on the detrostix reagent pad and inserted into the microprocessor of digital blood glucometer, and the reading was noted. After 72 hr, rats having BGL beyond 150 mg/dl of blood were selected for the study.

## 5. Effect of Alcoholic Extract of *Artemisia Maritima* Linn. Flower Heads on Alloxan-induced Hyperglycemia

### 5.1 Acute Treatment

During acute treatment, blood samples were collected from the tip of the tail just prior to and 1 and 3 hr after the extract/drug administration.

### 5.2 Sub-acute Treatment

During sub-acute treatment, all the test samples were administered daily, and the treatment period for all these groups was for 14 days. Blood samples were withdrawn from the rats by tail vein puncturing with hypodermic needle at 0, 7th and at the end of 14th day.

## 6. Statistical Evaluation

Evaluation was done by two-way-analysis of variance (ANOVA) followed by Bonferroni post test; $n$=6 in each group. $p$<0.001 and $F$=12 (acute) and 122 for (sub acute), which is significant.

## 7. Results

During acute study of the ethanolic extract of flower heads of *Artemisia maritima* at different treatment times showed statistically significant reduction in blood glucose level ($p$<0.001) at 3 hr after alloxan administration (Table 2). During subacute study, the oral administration of the ethanolic extract of flower heads of *A. maritima* at the doses of 250 mg/kg and 500 mg/kg body weight reduced significantly ($p$<0.001) the blood glucose level

**Table 1:** Effect of ethanolic extract of flower heads of *Artemisia maritima* on oral glucose tolerance test (OGTT)

| S. no. | Groups and doses (mg/kg, b.w) | | | Blood glucose level (mg/dl) |
|---|---|---|---|---|
| | 0 min | 30 min | 90 min | % Decrease at 90 min |
| 1. Glucose control | 72.0±0.9 | 250±0.9 | 140±0.9 | ---------- |
| 2. Glimepride (150) | 70±0.9 | 145±0.9 | 78±0.9 | 79.4% |
| 3. Flower heads extract (250) | 68±0.9 | 229±1.3*** | 135±0.9*** | 3 .7% |
| 4. Flower heads extract (500) | 71±0.9 | 226±1.3*** | 110±0.9*** | 27.2% |
| 5. Insulin (3 I.U/kg) | 75±0.9 | 130±0.9 | 72±0.9 | 94.4% |

Data are expressed as mean±SEM, $n$=6; evaluation by two-way analysis of variance (ANOVA);
$p$***<0.001 compared to glucose control, which is significant.

**Table 2:** Effect of acute treatment of flower heads of *Artemisia maritima* on blood glucose level in alloxan induced diabetic rats

| S. no. | Groups and doses (mg/kg, b.w) | | | Blood glucose level (mg/dl) |
|---|---|---|---|---|
| | 0 min | 1 hr | 3 hr | % Decrease at 3rd hr |
| 1. Normal control | 73.3±1.4 | 75.1±0.8 | 73.3±0.49 | ---------- |
| 2. Diabetic control | 169±2.6 | 162±0.7 | 170±1.3 | ---------- |
| 3. Glimepride (150) | 173±2.4 | 164±0.8 | 151±0.9*** | 12.5% |
| 4. Flower heads extract (250) | 172±2.2 | 169±.1.4* | 164±0.8*** | 3.6% |
| 5. Flower heads extract (500) | 170±4.4 | 164±0.8 | 151±0.2*** | 12.5% |
| 6. Insulin (3 I.U/kg) | 169±3.6 | 159±1.4 | 139±1.3 | 22.03% |

Values are mean±SEM for six observations, $n$=6; *$p$<0.05; ***shows $p$<0.001 compared to diabetic control, which is significant.

**Table 3:** Effect of sub acute treatment of alcoholic extract of *Artemisia maritima* on alloxan-induced diabetic rats

| S. no. | Groups and doses (mg/kg, b.w) | | | Blood glucose level (mg/dl) |
|---|---|---|---|---|
| | 0 day | 7th day | 14th day | % Decrease |
| 1. Normal control | 73.3±1.4 | 75.3±1.5 | 76.8±1.3 | ---------- |
| 2. Diabetic control | 169±2.6 | 197±5.4 | 230±8.9 | ---------- |
| 3. Glimepride (150) | 173±2.4 | 80.5±0.7*** | 73±1.5*** | 57% |
| 4. Flower heads extract (250) | 172±2.2 | 129±2.3*** | 88.3±2.4*** | 40% |
| 5. Flower heads extract (500) | 170±4.4 | 118±1.5*** | 81±0.4*** | 49% |
| 6. Insulin (3 I.U/kg) | 169±3.6 | 78.8±1.2 | 73±1.2 | 57% |

Values are mean±SEM for six observations, $n$=6; ***shows $p$<0.001 compared to diabetic control.

on 14th day after the administration of alloxan (Table 3). The ethanolic extract of *A. maritima* showed better anti-diabetic effect at a dose of 500 mg/kg that showed 49% of reduction in blood glucose level in comparison to a dose of 250 mg/kg that showed 40% of reduction in blood glucose level on 14th day after the administration of alloxan. The oral glucose tolerance test also showed significant decrease ($p$<0.001) after the treatment at different interval of time with glucose (Table 1).

## 8. Conclusion

The results indicated that ethanolic extract of flower heads of *Artemisia maritima* possess significant hypoglycaemic activity during both acute and sub-acute treatments. However, the ethanolic extract of flower heads of *Artemisia maritima* at a dose of 500 mg/kg body weight showed better efficacy than that with the 250 mg/kg body weight.

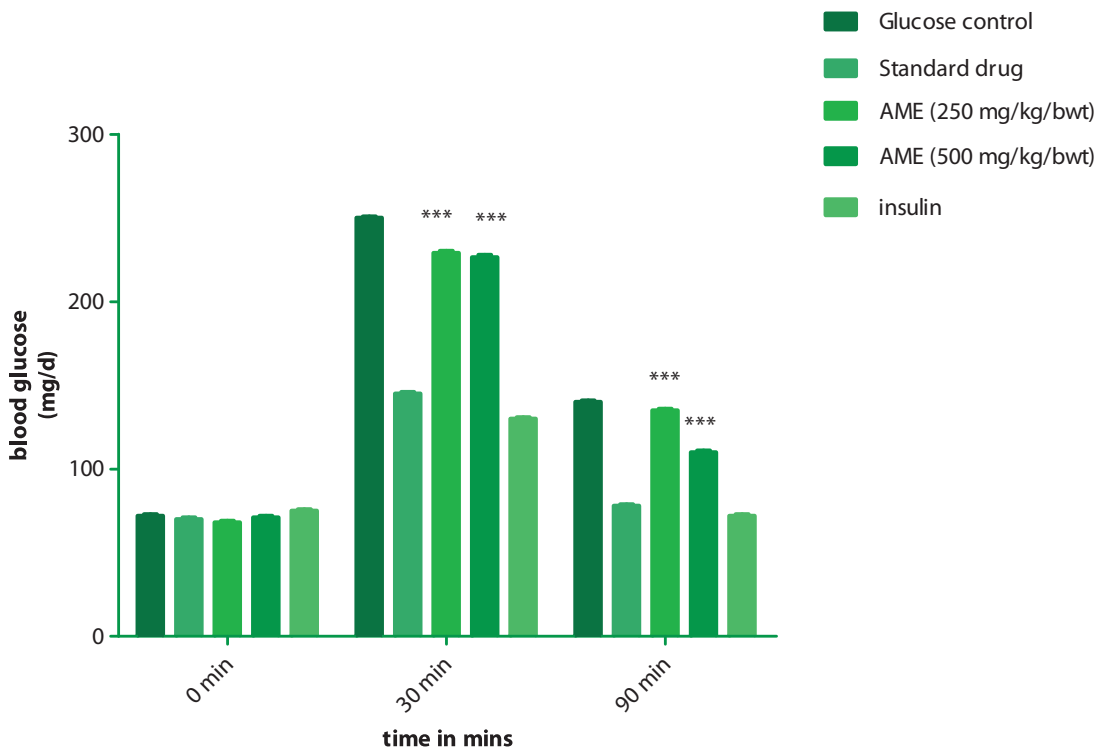


**Fig. 1.** Graph showing the blood glucose level (mg/dl) of different groups of rats at 0, 30 and 90 min after different treatment time.

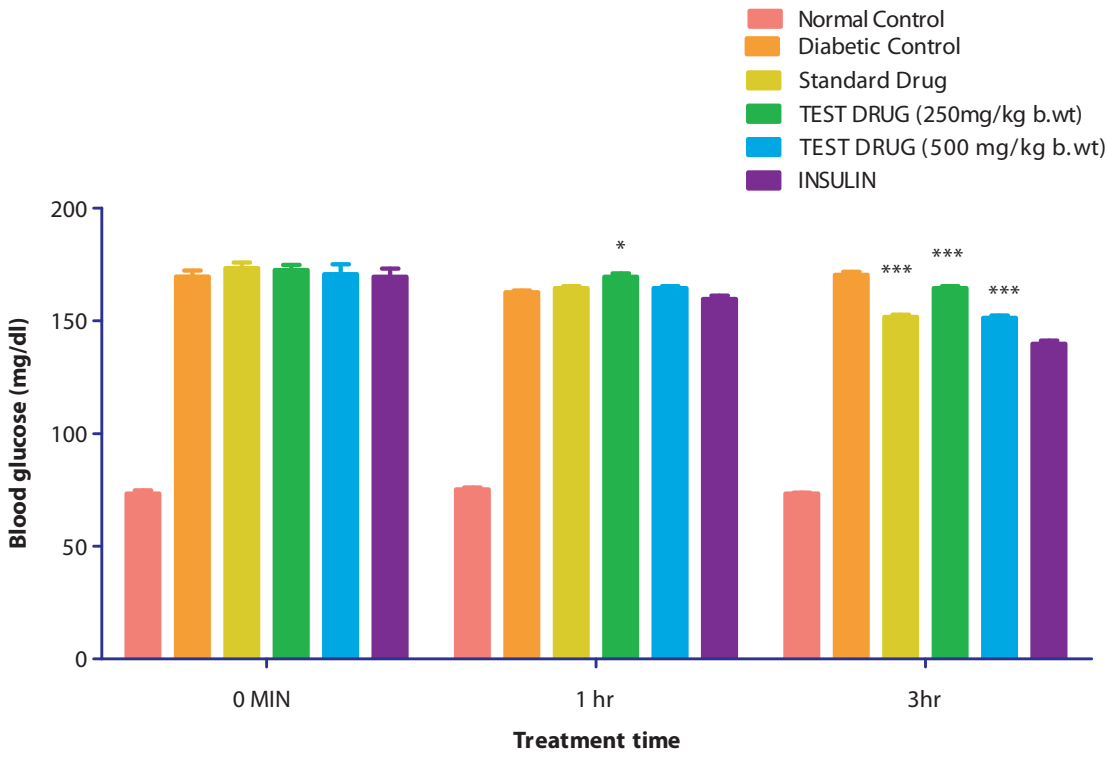


**Fig. 2.** Graph showing the blood glucose levels (mg/dl) of different groups of rats at 0, 1 and 3 hr after different treatment time intervals.

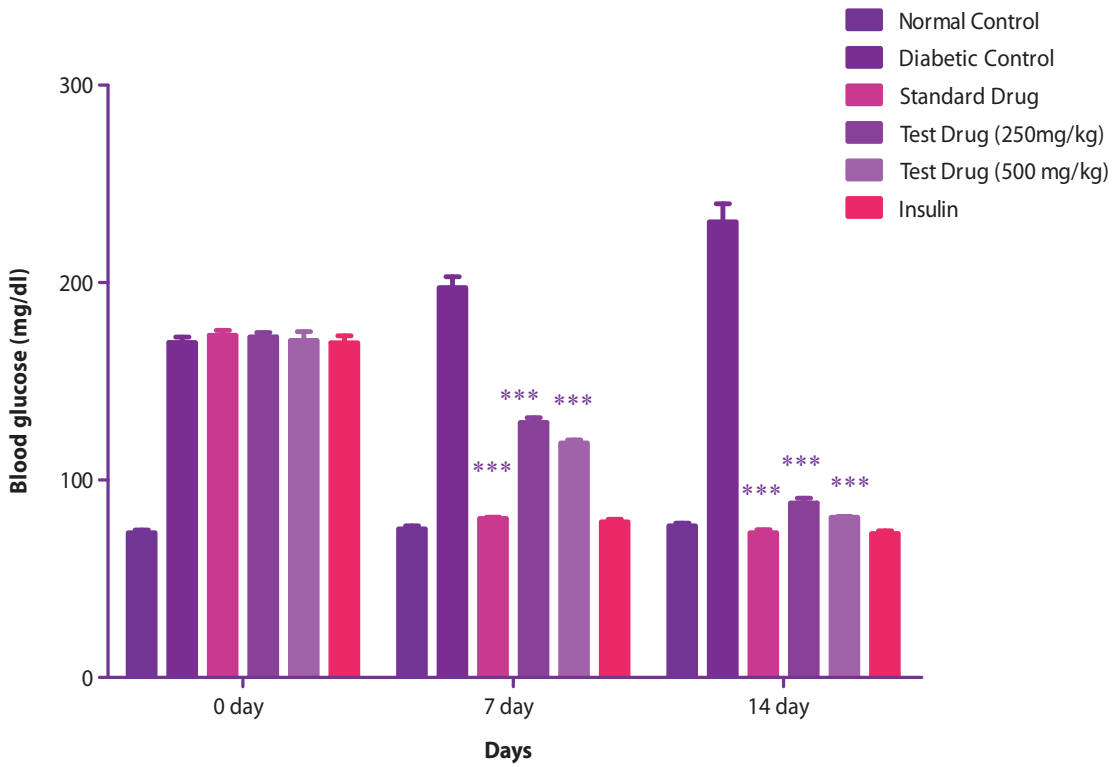


**Fig. 3.** Graph showing the blood glucose levels (mg/dl) of different groups of rats on 0, 7 th and 14 th days after different treatment time intervals.

## 9. Discussion

Alloxan produces oxygen radicals in the body, which causes pancreatic injury [15] and could be responsible for increased blood sugar seen in animals. It is generally accepted that alloxan treatment causes permanent destruction of β-cells [16]. It is, therefore, conceivable that the hypoglycaemic principles in the ethanolic extract of flower heads of *Artemisia maritima* may exert their effect by an extra pancreatic mechanism in diabetic rats. However the exact mechanism is not known, and further studies are under investigations.

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