

**HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITY OF AQUEOUS EXTRACT OF *DIOSPYROS PEREGRINA* FRUITS IN NORMAL AND ALLOXAN-INDUCED DIABETIC RABBITS**

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**Summary**

The study was conducted to evaluate hypoglycemic and antihyperglycemic activity of aqueous extract of matured fruits of *Diospyros peregrina* (AED) following oral administration (100, 200 and 300 mg/kg body weight) to normal and alloxan-induced (100 mg/kg body weight, i.v) diabetic rabbits. Blood samples were collected from the marginal ear vein before and also at 4, 6, 8, 10, 12, 16, 18, 20 and 24 h after drug administration and blood glucose levels were estimated by GOD/POD method using commercial glucose kits. The data was compared statistically by using student's t-test. The AED produced dose-dependent reduction in blood glucose of both normal and diabetic rabbits and comparable with that of the standard drug glibenclamide. The results indicate that, the AED possesses significant antidiabetic activity and supports the traditional usage of the matured fruits for the control of diabetes.

**Key words:** Diabetes, *Diospyros peregrina*, Alloxan monohydrate, Hypoglycemia, Antihyperglycemia

## Introduction

Diabetes mellitus is a global disease, found in all nations of the world and it is becoming a serious threat to the health of mankind and third killer of the human beings after cancer, cardiovascular and cerebrovascular diseases (1). It has been estimated the number of diabetes in India is expected to increase 57.2 million by the year 2025 (2). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with antidiabetic activity (3). In folk/tribal medical practice many plants are used to treat diabetes mellitus in India. Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side effects and relatively low cost (4). Most of these plants are not significantly validated for their therapeutic efficacy and safety. Scientific study on these plants is likely to provide invaluable antidiabetic drugs (5).

*Diospyros peregrina* Gurke (Family: Ebenaceae) is a small middle- sized tree and ripe fruits are edible with ethnomedicinal significance as tonic and aphrodisiac (6). The fruits contain alkenes, flavanoids, triterpenes and tannins (7). The fruits are used for the treatment of diarrhoea, dysentery, cholera, mouth ulcers and in wounds (8). The stem bark and matured fruits are used as drugs by the traditional medical practitioners in India (7) with beneficial action. Earlier reports indicate significant blood glucose lowering activity with methanolic extract of matured fruits of *Diospyros peregrina* in rats (9). Generally the use of aqueous extract of *D. peregrina* has been in practice by Ayurvedic physicians in India with beneficial action. Hence, in the present study the aqueous extract of *D. peregrina* has been evaluated for hypoglycemic activity in normal and alloxan-induced diabetic rabbits.

## Material and Methods

### Plant Material:

Matured unripe fruits of *Diospyros peregrina* were collected, identified and authenticated by botanist in Roland Institute of Pharmaceutical Sciences, Berhampur, India. A voucher specimen (F-009/2008) was deposited in the herbarium of our institution for future reference.

### Preparation of aqueous extract:

Matured unripe fruits of *Diospyros peregrina* were dried in an incubator for two days at 40°C, crushed in a mechanical grinder to fine powder. 500 g of the powder was mixed with 1000 ml of distilled water in a flask and boiled for 1 h. Following cooling at room temperature, the brew was filtered using Whatman No.1 filter paper.

The filtrate was then concentrated in a vacuum rotary evaporator (yield (%): 10.3 w/w) and the extract (AED) was stored at 4°C until required. Preliminary phytochemical screening (10) of aqueous extract of fruits revealed the presence of tannins, flavanoids, triterpenoids and sugars.

**Chemicals:**

Glibenclamide was provided as a generous gift sample by Hoechst Pharmaceuticals, Mumbai, India and alloxan monohydrate was purchased from LOBA Chemie, Mumbai, India. Glucose kits (Span diagnostics) were purchased from the local pharmacy. Blood samples were analyzed by using semiautoanalyser (Screen Master 3000, Mumbai, India). All other chemicals used for this study were analytical grade.

**Animals:**

Adult albino rabbits of either sex weighing between 1.5-2.0 kg were procured from M/s. Ghosh Enterprises, Kolkata, India were used in the study. They were maintained on standard diet and water *ad libitum*. All the rabbits were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in a well-ventilated animal house with a 12-h light/12-h dark cycle. They were fasted for 18 h prior to the experiment (allowing access to water) and, during the experiment, food and water were withdrawn. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Roland Institute of Pharmaceutical Sciences, Berhampur, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All studies were carried out using 6 rabbits in each group.

**Study in normal rabbits:**

Group-I/II/III/IV/V were treated orally with distilled water/AED-100 mg/kg body weight/ AED-200 mg/kg body weight/ AED-300 mg/kg body weight/Glibenclamide-40 µg/kg body weight respectively.

**Study in diabetic rabbits:**

These groups were rendered diabetic by injecting alloxan monohydrate (100 mg/kg body weight i.v) in to the marginal ear vein after base line blood glucose estimation was done. After two weeks when the condition of diabetes was stabilized, rabbits with blood glucose levels above 300 mg/dl were selected for the study. These diabetic rabbits were divided in to 5 groups and treated as mentioned above.

**Collection of blood samples:**

Blood samples (appr. 0.3 ml) were collected by puncturing the marginal ear vein of each rabbit of a group before and also at 4, 6, 8, 10, 12, 16, 18, 20 and 24 h after oral drug administration. Blood glucose levels were determined by using GOD-POD method (11).

**Statistical analysis:**

Data were expressed as mean  $\pm$  SEM. Statistical analysis was made by using student's unpaired t-test.

**Results****Effect in normal rabbits:**

The AED produced a dose-dependent hypoglycemia in normal rabbits. It produced maximum reduction in blood glucose of 17.7% (6 h,  $p < 0.05$ ), 29.6% (18 h,  $p < 0.05$ ) and 32.9% (20 h,  $p < 0.01$ ) with doses of 100, 200 and 300 mg/kg body weight respectively (Table 1). Glibenclamide-40  $\mu$ g/kg body weight produced significant reduction in blood glucose ( $p < 0.01$ ) compared to control (32.9%, 8 h).

**Table 1: Percentage blood glucose reduction in normal rabbits (n=6)**

Time (h)	Control	AED (100 mg/kg)	AED (200 mg/kg)	AED (300 mg/kg)	Glibenclamide (40 $\mu$ g/kg)
Initial blood glucose (mg/dl)	106.6	100.2	102.4	109.5	105.2
4	-4.1 $\pm$ 6.3	12.5 $\pm$ 1.7*	14.9 $\pm$ 6.1*	15.1 $\pm$ 4.3*	26.5 $\pm$ 5.1**
6	-3.4 $\pm$ 7.4	17.7 $\pm$ 2.2*	18.1 $\pm$ 2.9*	18.5 $\pm$ 4.2*	30.7 $\pm$ 5.9**
8	-1.4 $\pm$ 7.2	16.3 $\pm$ 4.1	20.5 $\pm$ 4.4*	20.2 $\pm$ 3.6*	32.9 $\pm$ 5.8**
10	-4.6 $\pm$ 6.0	13.6 $\pm$ 3.4*	20.7 $\pm$ 6.7*	21.8 $\pm$ 7.1*	30.8 $\pm$ 6.2**
12	-7.9 $\pm$ 7.3	5.9 $\pm$ 9.0	23.8 $\pm$ 4.1**	25.4 $\pm$ 5.4**	29.3 $\pm$ 5.7**
16	-5.5 $\pm$ 5.2	5.6 $\pm$ 7.6	28.0 $\pm$ 1.7***	30.6 $\pm$ 5.2***	24.1 $\pm$ 4.6**
18	-1.4 $\pm$ 10.9	9.1 $\pm$ 3.1	29.6 $\pm$ 1.4*	31.7 $\pm$ 6.2*	22.9 $\pm$ 4.6
20	6.9 $\pm$ 2.5	9.9 $\pm$ 2.9	15.7 $\pm$ 7.2	32.9 $\pm$ 6.3**	20.8 $\pm$ 6.4
24	-2.6 $\pm$ 5.6	2.0 $\pm$ 5.4	5.3 $\pm$ 8.1	31.0 $\pm$ 2.4***	18.3 $\pm$ 5.7

AED: Aqueous extract of matured fruits of *Diospyros peregrina*

Significant difference from control at corresponding intervals: \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$

**Effect in diabetic rabbits:**

Dose-dependent reduction in blood glucose was also observed in alloxan-induced diabetic rabbits treated with AED. The percentage reduction in blood glucose tended to be higher in the diabetic condition compared to the normal state. A significant reduction ( $p < 0.001$ ) in blood glucose of 20.6% (8 h), 32.4% (18 h) and 37.5% (20 h) was observed with AED at a doses of 100, 200 and 300 mg/kg body weight respectively (Table 2). Glibenclamide produced a significant reduction ( $p < 0.001$ ) in blood glucose compared to diabetic control at 8 h (35.9%).

**Table 2: Percentage blood glucose reduction in diabetic rabbits (n=6)**

Time (h)	Control	AED (100 mg/kg)	AED (200 mg/kg)	AED (300 mg/kg)	Glibenclamide (40 µg/kg)
Initial blood glucose (mg/dl)	325.7	317.0	305.9	320.2	324.9
4	2.2 ± 1.3	11.5 ± 2.9*	15.6 ± 5.5*	17.8 ± 6.8*	23.6 ± 4.6***
6	1.7 ± 1.3	15.9 ± 1.8***	17.7 ± 3.5***	21.9 ± 6.5**	29.5 ± 6.5***
8	1.1 ± 1.3	20.6 ± 1.5***	23.2 ± 4.8***	25.1 ± 3.9***	35.9 ± 5.6***
10	-1.5 ± 1.4	17.8 ± 1.8***	25.5 ± 8.4**	27.6 ± 5.9***	29.7 ± 7.5**
12	-1.5 ± 1.2	14.4 ± 5.9**	28.8 ± 7.5**	28.5 ± 10.2*	25.8 ± 6.7**
16	1.2 ± 1.5	9.9 ± 2.9**	28.4 ± 4.5***	31.4 ± 7.9*	25.6 ± 5.1***
18	-1.6 ± 2.9	8.7 ± 3.4*	32.4 ± 6.6***	33.7 ± 7.0***	22.3 ± 6.6**
20	-1.7 ± 1.1	7.3 ± 2.3***	26.8 ± 6.1***	37.5 ± 5.8***	18.4 ± 10.8
24	-1.2 ± 1.3	5.4 ± 2.6*	20.4 ± 7.4*	32.8 ± 9.5**	15.5 ± 6.9*

AED: Aqueous extract of matured fruits of *Diospyros peregrina*

Significant difference from control at corresponding intervals: \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$

**Discussion**

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (12). Considerably large number of hypoglycemic/antidiabetic plants and herbs are known through folklore but their introduction in to modern therapy waits pharmacological testing by modern methods. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

Fruits of *Diospyros peregrina* are used traditionally by diabetic patients in India and are usually taken as aqueous extracts due to this reason the aqueous extract of this plant was evaluated and the data also confirmed the traditional indications. Earlier investigations

(9) on the antidiabetic activity of the organic extract of *D. peregrina* also substantiate the results of our studies in rabbits. Moreover, the fact that the extract has a more prolonged effect (at 300 mg/kg body weight) than the glibenclamide dose in the period of 18-24 h after treatment indicates a prolonged duration of antidiabetic action and could be due to multiple sites of action possessed by the active principles of *D. peregrina*.

Alloxan monohydrate, a beta-cytotoxin causes a massive destruction of  $\beta$ -cells of the islets of Langerhans resulting in reduced synthesis and release of insulin and has been reported to cause diabetes by damaging the pancreas due to free radical-related mechanisms (13). It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan-induced diabetes where as they are inactive in intense alloxan diabetes (nearly all  $\beta$ -cells have been destroyed) (14, 15). Since our results showed that glibenclamide reduced blood glucose levels in hyperglycemic animals, the state of diabetes is not severe. Alloxan treated animals receiving the AED showed rapid normalization of blood glucose levels in comparison to control and this could be due to possibility that some  $\beta$ -cells are still surviving to act upon by AED to exert its insulin releasing effect. Moreover, like sulphonylureas oral administration of AED produced hypoglycemia in normal animals. This suggests that the mode of action of the active ingredients of *Diospyros peregrina* is probably mediated by an enhanced secretion of insulin, like sulphonylureas. Further this plant possesses significant antioxidant potency and it may have some touch in antidiabetic activity. However, the possibility that enhanced tissue glucose utilization by *D. peregrina* cannot be ruled out. Preliminary phytochemical screening indicated the presence of flavanoids in the extract. Flavanoids indicated from different sources are reported to have antioxidant activity and antihyperglycemic activity (16) so the lead compound may be flavanoid. Further work is obviously required to fractionate, purify and identify the active aqueous principle(s) present in the fruits of *Diospyros peregrina* and to elucidate the precise mechanism(s) of its hypoglycemic effect.

Our study clearly indicated a significant antidiabetic activity with the aqueous extract of *Diospyros peregrina* fruits and supports the traditional usage of fruits by Ayurvedic physicians for the control of diabetes. Hence it might be help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of antidiabetic drugs.

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