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**Original Research Article**

# Anti-Diabetic Activity of Ethanolic Extract of *Nerium Oleander* Flowers in Alloxan Induced Diabetic Rats

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**Abstract:** The present study was aimed to evaluate the anti-diabetic activity of ethanolic flower extract of *Nerium oleander*. Hypoglycemic activity was carried on normal rats and antidiabetic activity on alloxan induced diabetic rats. The experimental group was rendered diabetic by I.P injection single dose of 150 mg/kg Alloxan monohydrate. Diabetic rats were initially treated orally with ethanolic flower extract of *Nerium oleander* at low dose of 200mg/kg and at high dose of 400 mg/kg and then compared with standard drug Glibenclamide at dose of 500µg/kg. Acute study for one day (0thhr, 1sthr, 2ndhr, 4thhr, 6thhr, 8thhr) and chronic study for 14 days (0th day, 7th day & 14th day) was carried out on alloxan induced diabetic rats using ethanolic flower extract of *Nerium oleander*. The effects of ethanolic flower extract of *Nerium oleander* on body weights in rats was also observed in chronic study. Lipid profile and biochemical parameters were evaluated. These results suggest that *Nerium oleander* has a beneficial effect on diabetes.

**Key Words:** Anti-diabetic, *Nerium oleander*, Glibenclamide, Alloxan monohydrate.

## Introduction

The term diabetes mellitus describes a metabolic disorder of multiple etiology which results in increased blood glucose levels and disturbances of carbohydrates, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (W.H.O 2006). Anti-diabetic drugs are medicines developed to stabilize and control blood glucose levels and thus manage diabetes (Yassin MM *et al.,* 2004).

*Nerium oleander* which is ornamental shrub belonging to family Apocynaceae. It is commonly called as Rosebay (or) oleander (or) soland. The chemical constituents that *Nerium oleander* contains glycosides, amino acids, steroids, flavonoids and tannins. *Nerium oleander* contains different pharmacological activities. Leaves and flowers have cardiotonic, diaphoretic, diuretic, anticancer, antibacterial, antifungal and expectorant activities (Prashanth Daware 2012). Root is a powerful resolvent applied to tumours (H.F.M. Ali *et al.,* 2012). Bark is used in febrifuge and intermittent fever. Flower part shows anticonvulsant activity (Poojasaini *et al.,* 2010). Seeds are poisonous (VeronikaBandara *et al.,* 2010) and abortifacient. The whole plant is said to have anti-cancer properties (Rashan LJ *et al.,* 2011). Glibenclamide is taken as a standard drug in this research work which is sulfonyl urea used in treatment of type-2 diabetes.

## Materials and Methods

**Plant Material:** The flowers of *Nerium oleander* was collected during Jan-March 2014 from Avanigadda, Andhra Pradesh, India. The flowers of *Nerium oleander* was identified and authenticated by the taxonomist Dr. T.V. Raghavarao, Maharani College, Peddapuram.

**Preparation of Extract:** The freshly collected flowers were cleaned from dirt and dried under shade for one week. The dried flowers are made in to coarse powder. The powder was macerated in ethanol for a period of 7 days and then subjected to hot percolation for 8 hrs. Then the solution was filtered, concentrated and then dried.

**Acute study in Normal Rats:** Adult Albino rats of either sex weighing between 160-250 grams were used for the study. Rats were maintained on uniform diet and at room temperature (Prabhakar Patil *et al.,* 2013). Animals were divided into 3 groups of 3 rats each.

**Group I:** Rats were served as normal control and received the vehicle orally.
**Group II:** Rats were administered with ethanolic flower extract of *Nerium oleander* 200 mg/kg in vehicle as orally
**Group III:** Rats were administered with ethanolic flower extract of *Nerium oleander* 400 mg/kg in vehicle as orally.

Blood samples were collected by tail vein puncture just prior to drug administration. After giving the drug solution, 0th hour blood

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glucose level should be checked. Later on, at frequent intervals blood glucose level should be checked at 0th, 1st, 2nd, 4th, 6th, 8thhr intervals using glucometer (Om PrakashBangar *et al.,* 2009).

**Method of antidiabetic activity:**

**Experimental animals:** Adult albino rats of either sex weighing between 160-200gms were used for the study. Rats were maintained on uniform diet and at room temperature with 12 h/12 h light and dark cycle (Kar A *et al.,* 2003). They were housed in polypropylene cages. Rats were fed with standard animal pellet diet and water.

**Induction of diabetes:** The animals were fasted for 18hrs and diabetes was induced by a single intra peritoneal injection of a freshly prepared solution of alloxan monohydrate (150 mg/kg) in ice cold 0.9% saline (NaCl) solution (Hossein Zadeh H *et al.,* 1998). The animals were given 2ml of 5% dextrose solution using orogastric tube immediately after induction to overcome the drug induced hypoglycemia. Seventy-two hours later, rats with blood glucose levels (BGLs) above 200 mg/dl were considered diabetic and selected for the experiment.

**Experimental design:** The animals were randomly divided into five groups with 6 rats in each group and treated as follows:

**Group I:** Normal control rats;
**Group II:** Diabetic control rats;
**Group III:** Diabetic rats treated with flower extract at dose of 200 mg/kg
**Group IV:** Diabetic rats treated with flower extract at dose of 400 mg/kg
**Group V:** Diabetic rats treated with Glibenclamide at dose of 500µg/kg.

The change in body weight and fasting BGLs of all the rats were recorded at regular intervals during the experimental period. For acute study, the blood glucose levels were monitored after 1,2,4,6 and 8hrs of administration of a single dose of the extract and at the end of 0, 7 and 14 days for prolonged treatments. The blood glucose levels were monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on the accucheck glucose strip. This was inserted into digital blood glucometer and the readings were recorded. The collected blood was allowed to clot at room temperature and serum was separated by centrifugation at 3000 rpm for 10min. The serum was used for the estimation of biochemical parameters, lipid profile [Total cholesterol, high density lipoprotein (HDL), and Triglycerides] were analysed by auto-biochemistry analyser using standard ROBONIK laboratory kits.

## Results

By treating Normal rats with *Nerium oleander* flower extract, 28.95%and 35.5% reduction of blood glucose level has been observed at 6thhr interval. The significant percentage reduction in blood glucose levels for *Nerium oleander* flower extract in alloxan induced diabetic rats under acute study was 34.25% with low dose 200mg/kg at 6thhr interval where as 27.37% with high dose 400mg/kg at 4th interval has been observed. The percentage reduction in blood glucose levels for Glibenclamide (500µg/kg) was 41.53% at 4thhr interval was observed.

The significant percentage reduction in blood glucose levels for *Nerium oleander* flower extract in alloxan induced diabetic rats under chronic study was 9.8% on 7th day and 31.5% on 14th day with low dose 200mg/kg, where as 20.6% on 7th day and 40.95% on 14th day with high dose 400mg/kg has been observed. The percentage reduction in blood glucose levels for Glibenclamide (500µg/kg) was 30.5% on7th day and 52.27% on 14th day was observed. The marked decrease of total cholesterol was observed in diabetic treated rats with percentage reduction of 17.6% and 22.8% with low dose 200mg/kg and high dose 400mg/kg of *Nerium oleander* flower extract. There is further reduction in triglycerides with *Nerium oleander* flower extract (36.8% and 44.2%) was observed with low dose 200mg/kg and high dose 400mg/kg. The marked decrease in body weights in chronic study with Nerium oleander flower extract was also observed (Table 4).

**Table 1:** Variation in Blood glucose levels after oral administration of Ethanolic flower extract of *Nerium oleander* in normal rats (Acute Study)

| Treatment (mg/kg) | Changes in Glucose level (mg/dl) | | | | | |
|---|---|---|---|---|---|---|
| | 0 hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | 8 hr. |
| Group I: Normal (Control) | 93.9±1.6 | 92.0±1.5 | 90.4±1.2 | 88.0±1.2 | 83.0±1.26 | 85.0±1.2 |
| Group II: NOFE (200mg/kg b.w) | 105.0±6.8 | 98.6±9.4 | 94.6±9.6 | 79.6±9.2 | 74.6±7.8 | 76.0±6.5 |
| Group III: NOFE (400mg/kg b.w) | 93.6±3.9 | 73.0±14.4 | 67.3±13.2 | 65.0±8.5 | 60.3±3.7 | 65.3±3.4 |
| Group IV: Glibenclamide (500µg/kg b.w) | 91.6±0.9 | 70.7±2.1 | 68.0±1.5 | 64.3±0.9 | 59.6±0.9 | 61.5±0.7 |

**NOTE:** *Nerium oleander* flower extract

**Table 2:** Variation in Blood glucose levels after oral administration of Ethanolic flower extract of *Nerium oleander* in Alloxan induced diabetic rats (Acute Study)

| Treatment (mg/kg) | Changes in Glucose level (mg/dl) | | | | | |
|---|---|---|---|---|---|---|
| | 0 hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | 8 hr. |
| **Group I:** Diabetic (Control) | 351.6±0.88 | 350±0.57 | 350.3±0.33 | 349±0.57 | 350±0.6 | 348.6±0.9 |
| **Group II:** NOFE (200mg/kg b.w) | 313.33±5.46 | 305.00±5.14 | 287.00±7.01 | 231.00±5.51 | 206.00±3.06 | 240.00±5.78 |
| **Group III:** NOFE (400mg/kg b.w) | 319.00±5.51 | 288.33±4.41 | 268.33±4.41 | 231.67±9.29 | 253.33±4.41 | 278.33±1.67 |
| **Group IV:** Glibenclamide (500µg/kg b.w) | 330.67±8.12 | 308.33±6.02 | 281.67±4.41 | 224.33±3.48 | 193.33±6.02 | 241.00±2.08 |

**NOTE:** *Nerium oleander* flower extract

**Table 3:** Variation in Blood glucose levels after oral administration of ethanolic flower extract of *Nerium oleander* in Alloxan induced diabetic rats (Chronic Study)

| Treatment (mg/kg) | Changes in Glucose level (mg/dl) | | |
|---|---|---|---|
| | Initial Day | 7th day | 14th day |
| **Group I:** Normal (Control) | 75.4±1.87 | 83.3±1.53 | 84.3±2.03 |
| **Group II:** Diabetic (control) | 313±3.61 | 336.3± 5.13 | 359±3.00 |
| **Group III:** NOFE (200mg/kg b.w) | 313±4.58 | 282.3 ± 3.06 | 214.3±5.03 |
| **Group IV:** NOFE (400mg/kg b.w) | 316.7±1.70 | 251.3± 5.03 | 187±6.24 |
| **Group V:** Glibenclamide (500µg/kg b.w) | 308.3±6.51 | 214.3± 1.15 | 147.0±4.36 |

**NOTE:** *Nerium oleander* flower extract

**Table 4:** Effects of Ethanolic flower extract of *Nerium oleander* on body weights in rats

| Treatment (mg/kg) | Changes in Glucose level (mg/dl) | | |
|---|---|---|---|
| | Initial Day | 7th day | 14th day |
| **Group I:** Normal (Control) | 183.7±5.69 | 186.3 ± 5.13 | 191.3±3.21 |
| **Group II:** Diabetic (control) | 194±4.58 | 176.3 ± 1.53 | 172±3.00 |
| **Group III:** NOFE (200mg/kg b.w) | 195.3±3.06 | 200.7 ± 1.53 | 192.3±3.06 |
| **Group IV:** NOFE (400mg/kg b.w) | 201±3.61 | 197± 1.00 | 195.3±2.52 |
| **Group V:** Glibenclamide (500µg/kg b.w) | 193±7.00 | 189.3± 3.79 | 192.0±5.29 |

**NOTE:** *Nerium oleander* flower extract

**Table 5:** Lipid Levels with Standard and Extract in Alloxan Induced Diabetic Rats

| | Normal control | Diabetic Control | Diabetic +NOFE (200mg/kg) | Diabetic +NOFE (400mg/kg) | Diabetic +Glibenclamide (500µg/kg) |
|---|---|---|---|---|---|
| **Total cholesterol** | 52.33±2.5 | 115.33±2.5 | 95.00±4.58 | 89.00±1.00 | 89.00±3.61 |
| **Triglyceride** | 46.33±1.53 | 135.67±4 | 85.67±4.93 | 75.67±4.04 | 71.67±3.51 |

**NOTE:** *Nerium oleander* flower extract

## Discussion

The mode of action may by increasing the amount of insulin secreted by the pancreas (or) by increasing the sensitivity of target organs to insulin (or) decreasing the rate at which glucose is absorbed from the gastrointestinal tract (Kirkham. S *et al.,* 2009). *Nerium oleander* flower extract may show any of these above modes of action.

A marked increase in serum concentration of total cholesterol and triglycerides was observed with diabetic rats than normal control group which is often linked with hyperlipidaemia. During diabetic state, insulin deficiency contributes to de-arrangements of various metabolic and regulatory mechanisms in body (Raju. S *et al.,* 2012). At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids. Result of this study reveals that the administration of *Nerium oleander* flower extract lowered elevated blood glucose levels, body weights, total cholesterol and triglycerides.

## Conclusion

The study was performed to evaluate the anti-diabetic activity of ethanolic flower extract of *Nerium oleander*. The results obtained indicate the significant anti-diabetic activity and results were compared with standard reference Glibenclamide. This study also demonstrates significant lowering of lipid profile in alloxan induced diabetic rats. This is considered to be the most interesting aspect to explore and elucidate the possible mechanism responsible for the significant anti diabetic activity. Further research investigations may be required to isolate the actual phyto-constituents responsible for antidiabetic activity.

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