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# EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *Solanum Nigrum* IN ALLOXAN INDUCED DIABETIC RATS

**Yasmeen A Maniyar [1], Umamageswari M S[2*], Karthikeyan T M [3]**

[1, 2*] *Department of pharmacology S. Nijalingappa Medical College and H.S.K Hospital and Research Centre, Bagalkot, Karnataka, India*

[3] *Department of pathology, Melmaruvathur Adhiparasakthi institute of medical sciences and research, Melmaruvathur, Tamil Nadu 603319*

***Corresponding Author Email: umakarthikm@gmail.com**

## ABSTRACT

***Objectives:*** *Evaluation of antihyperglycemic activity of aqueous extract of leaves of Solanum nigrum (AELSN) in Alloxan induced diabetic rats. Comparison of antihyperglycemic activity of aqueous extract of leaves of Solanum nigrum with standard drug Glimepiride.* ***Methodology:*** *200-250 g of Wistar rats of either sex were divided into 4 groups (n=6). Group I is normal control (received 0.5ml normal saline), Group II is diabetic control (received 0.5ml normal saline), Group III is standard group (received glimepiride 0.1mg/Kg b.w.), Group IV is AELSN group (received AELSN 400mg/kg b.w.). Diabetes was induced by using Alloxal monohydrate 120mg/Kg b.w. intraperitoneally. All the animals received the respective drug for 21 days. Fasting blood glucose was measured by using the glucometer on 0 day, 1st day, 7th day, 14th day, & 21st day.* ***Results:*** *Aqueous extract of leaves of Solanum nigrum Linn showed significant fall in fasting blood sugar (p value < 0.01) at 21st day, but less than the standard drug Glimepiride (p value < 0.001). This study can be recommended for further evaluation.*

## KEYWORDS

Aqueous leaf extract, antihyperglycemic, *Solanum nigrum.*

## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus. Diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and Biguanides[1, 2, 3].

According to International Diabetes Federation the number of people with diabetes in India is currently around 40.9 million and is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken[4]. More than 1200 plant species are used worldwide in diabetes phytotherapy, and experimental study support the antihyperglycemic activity of a large number of these plants[5].

In recent years, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. *Solanum nigrum* is a medicinal plant. It has been used widely as medicinal and food plants despite their reputation for being poisonous. The unripe fruit of S. nigrum contains the highest concentration of toxin particularly Solanine[6]. The level of toxin in the berries is greatly reduced by ripening[7, 8]. *Solanum nigrum* (Family: Solanceae) is a shrub

found throughout India, which is traditionally used for inflammatory, diuretic and liver disorders[9]. The phytochemical studies revealed the presence of an alkaloid called solamargine, saponins[10].

## MATERIALS AND METHODS

**Plant Material:**

Leaves of *Solanum nigrum* were collected locally in Bagalkot district in the period of June to November 2011. Identity of the plant was authenticated by botanist Prof. Jadimath and voucher specimen was deposited in the Herbarium of Department of Pharmacology of S. Nijalingappa Medical College, Bagalkot, Karnataka, India.

**Preparation of the extract:**

The leaves of the plant were dried in shade. The dried leaves were reduced to a coarse powder by using dry grinder and passed through sieve. The powdered sample (50 g) was boiled in water for 30 min after which it was filtered using a piece of white cotton gauze. The filtrate was evaporated to dry at 40°C producing a brown color Solid residue obtained. The residue was weighed and stored in desiccators for the removal of remaining moisture. The final amount of Solid residue was 30% w/w.

**Chemicals:**

Glucometer (Acucheck-Sensor) was purchased from Roche Diagnostics which measures the blood glucose level by GOD–POD method (Glucose oxidase-peroxidase method) Mumbai, India. Metformin was obtained as gift sample from IPCA Laboratories, Mumbai, India. Alloxan monohydrate was purchased from Sigma chemicals, USA. Ethanol was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

**Animals:**

Adult male albino rats, weighing 150-200 g were used for this study. Animals were acclimated for 15 days in our disease free animal house prior to the start of the experiment. The animals were kept in clean and dry plastic cages, with 12 h light: 12 h dark cycle at 25±2$^0$C temperature and 45-60% relative humidity. Animals were given free access to standard feed and water ad libitum. For experimental purpose the animals were kept on overnight fasting but allowed free access to water. The Institutional Animal Ethics Committee approved the study and all the instructions given by our Animal Ethics Committee were followed throughout the experimentation.

**Phytochemical screening:**

The extract was subjected to various qualitative chemical test to know the constituents present, by using simple and standard qualitative methods described by Trease and Evans[11].

**Acute toxicity study:**

Albino mice of either sex weighing between 20-30 g were used. The animals were fasted over night. Acute toxicity study was performed according to OECD guidelines; method followed is according to number 425. It was found that tolerated dose was higher than 5,000 mg/kg body weight[12].

**Induction of diabetes:**

After overnight fasting Type2 diabetes was induced by single dose (120 mg/kg, b.w., i.p.) of alloxan monohydrate (Sigma Ltd., USA) dissolved in normal saline. After 1 h of alloxan administration, the animals were Fed on standard pellets and water ad libitum. The animals were stabilized for a week and those showing blood glucose level more than 250 mg/dL were selected for the study[13].

## EXPERIMENTAL DESIGN

After 12 hr fasting all the animals showing blood glucose level less than 100mg/dl were randomly divided in to 4 groups of 6animals per group. Group I served as normal control or nondiabetic group, was treated with 0.5mg/100g of vehicle

(2% gum acacia) orally. Group 2 served as untreated diabetic control received 0.5 mg/100 g of vehicle (2% gum acacia).Group 3 served as standard group was treated with 0.1 mg/kg/day of Glimepiride. Groups 4 served as test group and was treated with 400mg/kg/day of aqueous extract of leaves of *Solanum nigrum* (AELSN). All the drugs were given orally for 21 days. The fasting blood glucose level was measured by using glucometer on day 0,1,7,14,21 by using tail blood withdrawal technique after 12 hr. On 21st day all the animals were sacrificed and the pancreas was sent for histopathological examination.

**Histopathological examination:**

After 21 days experimental period and the last blood sampling, the whole pancreas was removed after sacrificing the animal and was fixed in 10% formalin for histopathological examination. Sections were cut and stained by hematoxylin and eosin (H&E) for histological examination[14].

**Statistical analysis:**

All the values were expressed as mean ± SEM. The results were analyzed for statistical Significance by using one-way ANOVA followed by Dunnett's test. $p<0.05$ was considered significant.

**RESULTS:**

Administrations of a single dose of extract (5000 mg/kg, b.w., and p.o.) did not produce any mortality. All 5 animals were alive, healthy, and active during the observation period of 14 days.

**Effect of AELSN on diabetic rats:**

The antihyperglycemic activity of AELSN on the fasting blood sugar level of diabetic rats is shown in **Table 1**. Standard group (Glimepiride 0.1mg/kg/day) decreased elevated blood glucose level significantly ($p<0.001$) as compared with the diabetic control from day 1 onwards and the blood glucose level on day 21 was 94.00 ± 1.265 mg/dl. Chronic treatment with AELSN in the dose of 400 mg/kg b.w., in alloxan-induced diabetic rats showed a highly significant ($P<0.001$) decrease in the elevated blood glucose level as compared with the diabetic control from day 7 onwards and the blood sugar level on day 21 is 151.67±3.333 mg/dl.

**Table: 1 Effect of AELSN on blood glucose level in Alloxan (120 mg/kg. i.p.) induced diabetes rats:**

| Treatment | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 7 | Day 14 | Day 21 |
| Normal control (Vehicle 2% gum acacia)) | 85.83 ±2.496 | 101.83±1.138 | 84.17±3.664 | 88.33±2.985 | 87.00±3.697 |
| Diabetic control (Alloxan (120 mg/kg i.p.) +Vehicle 2% gum acacia) | 323.00±13.767*** | 323.83±15.283*** | 319.33±13.346*** | 322.50±14.007*** | 319.83±12.831*** |
| Standard group (Alloxan (120 mg/kg i.p.) +0.1 mg/kg Glimipride) | 332.83±17.945 | 257.50±15.353*** | 131.50±7.320*** | 98.50±3.074*** | 94.00±1.265*** |
| Test group (Alloxan (120 mg/kg i.p.)+AELSN 400 mg/kg.) | 303.00±22.277 | 312.50±17.297 | 231.50±7.320*** | 191.67±4.485*** | 151.67±3.333*** |

**All values are expressed as mean±SEM ($n$=6), Group 2 was compared with group 1, Groups —3,4 were compared with group 2; *$p$<0.05, **$p$<0.01,p<0.001*** ; AELSN- Aqueous extract f Solanum nigrum**

**Histopathological examination of pancreas:**

Histopathological examinations of pancreas of all groups are shown in **Fig 1, 2, 3, 4**. Histopathological examination of normal healthy control group (**Fig: 1**) showed normal acini and normal cellular population of the islets cell of Langerhans. However, in the Alloxan only treated rats, there was extensive damage of the islets of Langerhans and they appeared to be irregular (**Fig: 2**). Treatment of diabetic rats with Glimepiride showed moderate expansion of cellular population and size of islet cells (**Fig: 3**). However, AELSN (400 mg/kg b.w.) treated-diabetic rats showed partial restoration of islets population and hyperplasia of islet cells (**Fig: 4**).

**Figure 1: Normal control group (Gum acacia) shows the normal pancreatic lobule with islets.**

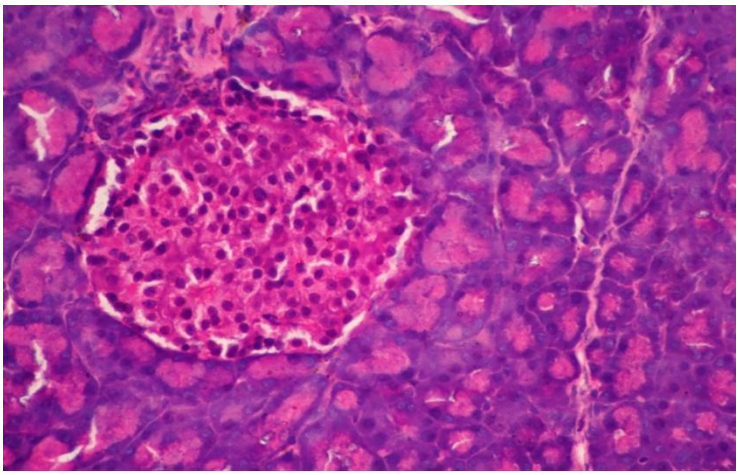


**Figure 2: Diabetic control group (Alloxan + gum acacia) shows the lobules devoid of islets.**

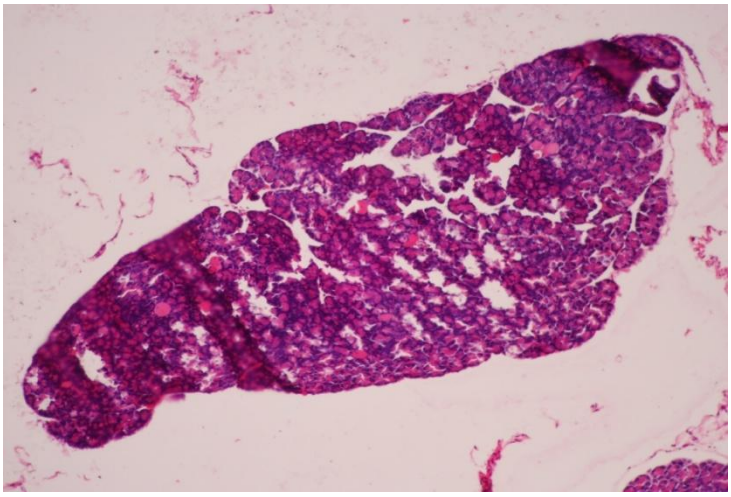


**Figure 3: Standard group (Alloxan + Glimepiride 0.1mg/kg b.w.) shows the lobule with regenerated islets.**

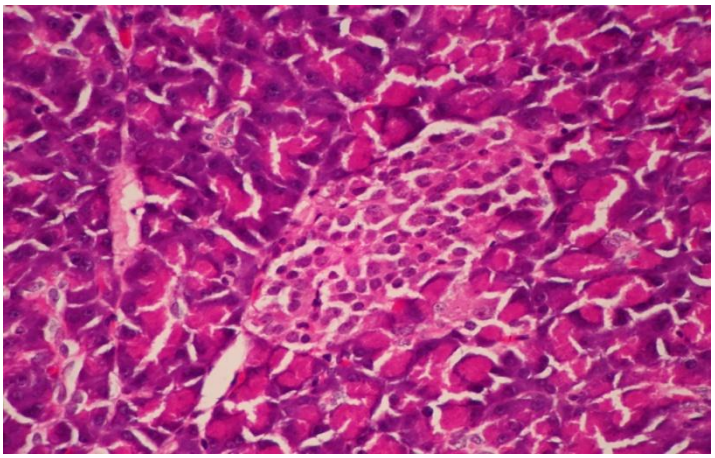
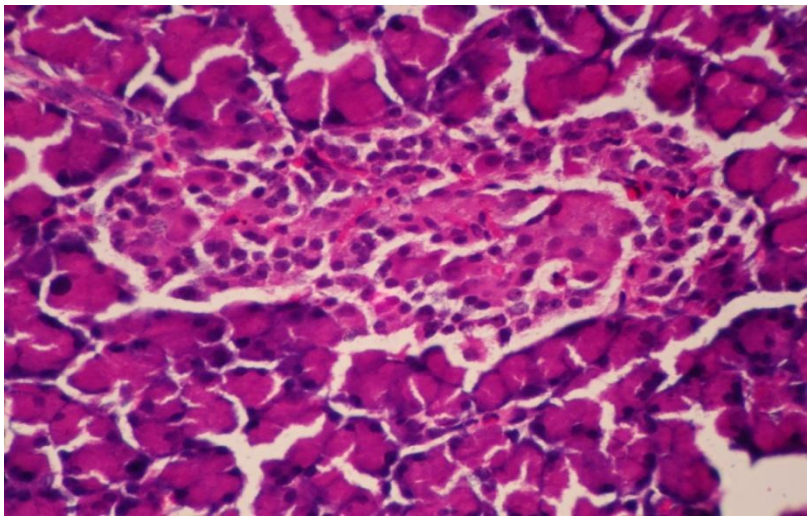


**Figure: 4: Test group (Alloxan + AELSN 400 mg/kg b.w.) shows the β cells with hyperplasia.**



## DISCUSSION

The present study shows the antihyperglycemic effect of AELSN in alloxan-induced diabetic rats. At the end of the study the percentage of reduction of blood sugar level with *Solanum nigrum* 400mg/kg/day b.w. is 52.6% and the percentage of reduction of blood glucose level with glimepiride 0.1mg/kg/day b.w. is 70.6% compared with diabetic control group.

Alloxan induces diabetes by destroying the insulin-producing beta cells of the pancreas[15]. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to induction of cell necrosis[16]. This action is mediated by reactive oxygen species with a simultaneous massive increase in calcium concentration leading to a rapid destruction of beta cells[17]. The use of low dose alloxan (120 mg/kg b.w.) produced partial destruction of pancreatic beta cells even though the animals became permanently diabetic[18]. Thus these animals have surviving beta cells and regeneration is possible[19].

Glimepiride, the second generation Sulfonylureas is known to mediate the antihyperglycemic effect by stimulating insulin release from pancreatic beta cells, reducing the hepatic clearance and suppressing the secretion of glucagon[20]. Sulfonylurea has been shown to suppress gluconeogenesis.

The antihyperglycemic effect of the aqueous extract may be due to the enhanced secretion of insulin from the beta cells of pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity.

The phytochemicals present in aqueous extract of *Solanum nigrum* are Alkaloids, Glycosides, Saponins, flavonoids, Tannins, and Sterols. Aali N S et al reported that antihyperglycemic activity of ethanolic extract of *Solanum nigrum* might be due to the presence of Saponins called Nigrumin I, Nigrumin II[21]. Flavonoids are potent antioxidants and are known to modulate the activities of various enzymes due to their interaction with various biomolecules. It was reported that flavonoids, alkaloids, tannins and phenolics possess bioactive antidiabetic activities [22, 23, 24].

In this studies, damaged pancreas was observed in alloxan-treated diabetic control rats (**Fig: 2**). The Glimepiride treated group showed regeneration of β-cells (**Fig: 3**). The comparable

regeneration was also shown by aqueous extracts of *Solanum nigrum* (**Fig: 4**). Histopathological examination shows healing of pancreas by AELSN, as a plausible mechanism of their antidiabetic activity. The antidiabetic activity of AELSN may probably be due to the presence of several bioactive principals like Flavanoids, Saponins, Alkaloids and Tannins.

## CONCLUSION

On the basis of the result in this experiment it may be stated that the aqueous extract of *Solanum nigrum* has a beneficial effect in diabetes mellitus. Further studies are required to purify the active principle and to study the molecular mechanism of the extract.

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***Corresponding Author:**
***Dr. Umamageswari M S,***
*Department of pharmacology,*
*SN Medical college, Bagalkot.*