THERAPEUTIC POTENTIAL OF HYDROETHONOLIC EXTRACT OF *NERIUMOLEANDER* FLOWERS FOR THEIR ANTIDIABETIC ACTIVITY

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ABSTRACT:

Diabetes Mellitus is a complicated metabolic illness that has greatly troubled human health and quality of life worldwide. Conventional methods are being used for treatment of diabetes; however they are not completely effective. These drugs maintain glucose level partially or temporarily. Medicinal plants with anti-hyperglycemic activities are being used at global level. In the present study the therapeutic potential of ethanolic extract of *Nerium oleander* flowers waschecked on diabetes *in vivo*. Alloxan was used to induce the diabetes in rabbits. Treatment rabbits were divided into two groups and given 200mg/kg body weight and 400mg/kg body weight per day respectively for 14 days. The decrease in blood glucose level was more significant in group II as compared to group I. Glucose level and histopathology was done to check the effect of extract of *N. oleander*.

Result showed decrease in blood glucose level in treatment group II (118.7 ± 7.4) and group I (150.64 ± 8.67) as compared to diabetic control (308 ±7.75). There was more pronounced decrease in blood glucose level in treatment group II as compared to group I. Serum insulin level was also improved in both treated groups i.e. in group I (7.67 ± 0.18) and group II (7.39 ± 0.9) versus diabetic controlled group (3.73 ± 0.26). Improvement in morphology of pancreas of treatment groups was observed. It was conclude that hydroethonolic extract of *N. oleander* flowers has tremendous effect against diabetes as it reduces the blood glucose level, improves glucose tolerance and also improves serum insulin level. the current study showed the hypoglycemic effect of ethanolic extract of *N. oleander.* OGTT was also performed and the results were quite satisfactory for treatment groups.

Key words*:* Diabetes mellitus, Glucagon likes peptide-1, Non-insulin dependent diabetes mellitus, Oral glucose tolerance test

INTRODUCTION:

Diabetes mellitus (DM) is a chronic disorder which is due to combination of hereditary and environmental factors. It is characterized by chronic hyperglycemia caused by deficiency in insulin action or secretion. It is found to damage most of the organ and tissue of body, such as circulatory system and nervous tissues (Cade, 2008). It is reported that more than 285 million people were affected with diabetes (Tariq *et al*., 2013) and diabetes will be the seventh leading cause of death in 2030 (WHO, 2011).

There are two major types of diabetes Type 1 diabetes (T1D) and Type II diabetes (T2D). T1D is associated with insulin deficiency by destruction ofpancreatic β- cells. T1D is normally due to autoimmune damage of β cells of pancreas (George *et al*., 2002). It can be managed with insulin injection. In T2D body cells fails to utilize insulin properly due to abnormal functioning of β-cells that leads to insulin deficiency and this condition called insulin resistance accompanied by decreased transport of glucose into fat cells and muscles due to which hepatic glucose output increased, all of which contribute to hyperglycemia(Setter *et al*., 2003). Over body weight and lack of physical exercise are major causes of diabetes. Serious health problems due to diabetes are cardiovascular disease, blindness, kidney failure and lower limb amputation.

Diabetes mellitus is main threat to the world. There are different methods and practices to cure diabetes, but all these methods are effective temporarily or partially. Oral administration of immunosuppressive drugs is frequently used to treat diabetes. These drugs cannot maintain the blood glucose level accurately. Furthermore these drugs are very expensive and also have side effects. In severe conditions insulin injections are given to the patient. Although glucose level can be controlled by using insulin, but there is a risk of hypoglycemia (Sexena and Kishore, 2004). It is very panic process. Moreover patients have to monitor blood glucose level several times in a day.

Another treatment strategy is β cell or stem cell transplantations. Islet/β- cells transplantation is the only successful therapy currently available (Tariq *et al.*, 2013). Stem cell therapy is potential replacement of β cell transplantation, but this 2 strategy is very

expensive and is still in its initial stages may be available in near future. Another possible cure that may come in future is the use of nanoparticles.

Currently there is rapid interest in herbal remedies due to side effects related with therapeutic agents such as oral hypoglycemic agents and insulin for the treatment of diabetes mellitus (Khurshid *et al*., 2012). Herbal and natural therapies are considered more safe and useful, and less expensive as compared to synthetic drugs.

Herbal medicine treatment is oldest form of health-care known to humans. Phytomedicines have great potential and had been used by all civilization throughout history. It was a basic part of the development of modern cultures/ civilization. Ethno botany studies the complex relationship between plants and cultures. It is multidisciplinary science defined as interaction between plants and people. The primitive man systematically collected information on the herbs and developed well-defined herbal drugs. Many drugs that are commonly used by humans today are of herbal origin. Most of drugs contain at least one active ingredient derived from plant extracts.

N. oleander is evergreen shrub and is widely used as a medicinal plant for many years in the treatment of different diseases. Some studies have investigated its toxicity and its cure action in diluted preparations (Haeba*et al.*, 2002).

N. oleander produces secondary metabolites; some of them have pharmacological interest. The important pharmacological activities are antifungal(Derwic *et al.*, 2010), antimicrobial (Hussain and Gorsi, 2004), antitumor (Ali *et al*., 2010) and anti-inflammatory and anti-nociceptive (Erdemoglu*et al.*, 2003).Although *N. oleander* has great effects against different diseases but still there is alack of knowledge about antidiabetic activity of *N. oleander*. Few studies havebeen conducted on *N. oleander* to explore its anti-diabetic potential. Therefore, thepresent work will provide some novel aspects about *N. oleander* and in the field oftraditional medicine.

The present study was designed to explore anti-diabetic potential of hydro ethanolic extract of *N. oleander* flower in drug induced animals. The *N. oleander* plant has been used traditionally as folk remedies for wide ranges of maladies and conditions including dermatitis, eczema, sores, abscesses, warts, skin cancer, asthma, heart tonics, and epilepsy. Anti-diabetic activity evaluation of *N. oleander* extract on insulin, glucose level and some liver enzyme activities was used by

Yassin and Mwafy, (2007). To check the therapeutic potential of *N. oleander* diabetic models were prepared by intravenous

administration of alloxan. Hydroethanolic extract of *N.oleander flower* was orally administered to diabetic animals. The dose of *N.oleander* extract was based on toxicological studies (Haeba *et al*., 2002; Adam *et al*., 2011). Their blood glucose level was checked every day and serum insulin was also checked. Moreover histopathology of pancreas was also done to check the effect of *N. oleander* flowers extract.To evaluate the efficacy of hydroethanolic extract of *N. oleander* againstalloxan induced diabetic model.

1. To identify and extract of plant material from *N. oleander*.2. To induce diabetes in rabbits.3. To asses therapeutic potential of extract of flowers of *N.* oleander after treatment.4. To study histopathology of pancreas.

MATERIAL AND METHODS:

Sampling and Collection of Plants Material

The samples of plant were collected from local areas of Mirpur AJK andKasguma. The collected Samples were identified by an expert botanist Dr. RehanaAsghar, Chairperson of Biotechnology Department, MUST AJK.

Extract Formation from Flowers*:*

After collection of plants flowers were separated, washed and were dried inshady places for ten days. Later flowers were grounded and then dip its powderform in 500ml solution containing 70:30 of ethanol and water respectively. Thesolution was suspended in Soxhlet apparatus. By heating solvent was evaporated,transformed into condenser where it was converted in liquid and was collected inextraction chamber. The solid material was slowly filled with worm solvent. Whenthe Soxhlet chamber was almost full it was automatically emptied, with solventrunning back to the distillation flask. The above cycle was repeated several timesin a day. The filtrate was concentrated and solvent was recovered using rotaryevaporated. The paste was obtained and weighted by means of electrical balance.The whole material was placed in refrigerator to avoid any type of contamination.

Figure 1: Soxhlet Apparatus

Experimental Animals Used

Inbred male domestic rabbits (*Oryctolaguscuniculus*) with weight of 1-2kg approximately from Mirpur and surrounding areas were used. They werebrought three months prior to

start of research in order to minimize the stresseffect. These rabbits were provided with natural habitat fresh water, vegetablegrasses, maize and some amount of glucose was also provided to these animals.The rabbits handled and treated carefully. The cages of these animals were cleanedon daily bases, fresh air and light was provided to these animals with reasonabletemperature.

Acute Toxicity Testing of Extract

For acute toxicity testing experimental animals were fasted overnight andwere provided only water and then the extract was given to the respective groups orally at the dose level of200mg/kg body-weight through digestive tract. Thesegroups were continuously observed for 24 hours for their behavior, neurologicaland autonomic profiles and then were studied for 72 hours in a week to check anytype of lethality. According to the guidelines if mortality is observed in 2 to 3animals, then the dose provided is toxic dose. If mortality is observed in oneanimal then the same dose is repeated to confirm the toxicity of that particulardose. If mortality is not observed at all, the plant extract is considered as non-toxic.No mortality was observed in experimental animals.

Induction of Diabetes in Rabbits

A group of rabbits with weight 1-1.5 kg were selected for alloxan injectionintravenously. 85mg/kg of alloxan was dissolved in 0.9% normal saline for makingit dilute solution. This solution was injected in jugular vein of rabbits with the helpof 3cc BD syringe. Winter green oil and a vasodilator methyl salicylate wereapplied to jugular vein before injection to make vein swell and prominent. The20% glucose in water was provided orally by means of small bottles for one day.The aim of glucose supply to animals was done to prevent them fromhypoglycemic shocks. The blood glucose level was routinely observed for five days with the help of glucometer. The test animals with blood glucose level more25than 250 mg/dl were considered as diabetic. At least two times the blood glucoselevel greater than 250mg/dl confirms animals as diabetic model.

Experimental Design

The research animals were divided into five groups

Normal Group: Given no alloxan and no treatment

Diabetic Group: Given alloxan but no treatment

Controlled Group: Given alloxan and treated with insulin @ 5 IU/kg/day

Treatment Group1: Diabetic animals treated with 200mg/kg *N. oleander*flower *e*xtract.

Treatment Group 2: Diabetic animals treated with 400mg/kg of *N. oleander*flower *e*xtractThe animals of different groups were tagged for their easy recognitionduring study. The above mentioned groups were provided with vegetables, fruitsand glucose during experimentation of three weeks.

3.7: *Treatment of diabetic animals:*

In each group 3 animals were used for research work. Group 1 normal andgroup 2 diabetic were not provided treatment. Group 3 animals diabetic wereprovided with daily insulin of 5IU/kg group 4 animals were treated with 200mg/kgweight extract of *N. oleander* flower per day through oral route, per day. Thetreatment was started after a week on those animals which had

hypoglycemicconditions. Flower extracts was present in the form of semisolid gel likeappearance. Before administration, extract was mixed with distilled water to makeit dilute. A dropper and food pipe was used to transfer the extract into mouth of therabbit. The procedure was repeated daily for two weeks and blood glucose levelwas checked on daily basis.

Measurement of Blood Glucose Level during Treatment

Blood glucose level of all experimental groups was monitored by glucoseoxidase method using Glucometer and standard glucose strips of ABBOT Ltd. Theblood glucose level was checked at every day after the start of treatment. Thenormal group was also detected at different times to observe any type of change inblood glucose level.

Measurement of Serum Insulin Levels*:*

Animals were handled with great care and blood samples were collectedimmediately from jugular vein. The jugular vein was then pressed with cottonsoaked with spirit to save the rabbits from infection.Samples of all model groups were collected after at the 10th day oftreatment in order to study any type change in pancreatic beta cells in to secreteinsulin. Serum was separated from the samples and insulin level was measured byELISA.

Oral Glucose Tolerance Test

Oral glucose tolerance test was applied to both diabetic controlled andtreated models of rabbits with *N. oleander*. The aim of this test was to observefailure of diabetic animal to metabolize the orally given glucose, and also observethe effect of glucose homeostasis in diabetic treated animals with *N. oleander*flower extract 200mg/kg and 400mg/kg body-weight. The rabbits were kept infasting condition for 12 hours and before giving them glucose solution, theirbaseline glucose level was checked with glucometer. The glucose solution wasthen provided to these rabbits orally 3g/kg their body weight with the help ofdropper. the blood was withdrawn from ear veins after 30 minutes, 60 minutes, 90minutes and 120 minutes of glucose administration via glucometer the glucoselevel for all groups was measured and results were compared of controlled andtreated animals.

3.8: Chemicals and Apparatus Used*:*

1. Rotary evaporator(Heidolph)
2. Refrigerator
3. Grinder machine
4. Graduated beakers
5. Filter paper
6. Ethanol
7. Alloxan monohydrate (Sigma Aldrich chemical, Saint Louis, MO,USA)
8. Glucose
9. Insulin
10. Weighing balance
11. Glucometer and standard glucose strips of ABBOT Ltd.
12. Glucose measuring strips
13. Soxheletapparatus(set 55/50pcs by Laboy)
14. Urine Strips from Bayers Diagnosis Ltd.

Statistical Analysis:

The data obtained from various experiments was recorded and wasevaluated statistically using one way analysis of variance (ANOVA); mean

valueswere determined along with standard deviation. Level of significance was kept asP>0.05.

RESULTS AND DISCUSSION

Comparison of Blood Glucose Levels of Normal Verses Diabetic Rabbits:

To induce diabetes in rabbits, they were induced with alloxan @ 85mg/kgbody weight. These rabbits showed symptoms of diabetes like polyurea with 3-4days. Diabetic status was confirmed by measurement of blood glucose level atfourth day alloxan induction by using ABBOT Glucometer. Rabbits with bloodglucose level > 250 mg/dl were confirmed to be diabetic. To establish a base linevalue of blood glucose in normal and diabetic rabbits, the blood glucose level oftwo groups was monitored for 14 days. The measurements were taken on dailybases. The results showed that rabbits induced alloxan have elevated level of bloodglucose as shown in Figure 4.1.

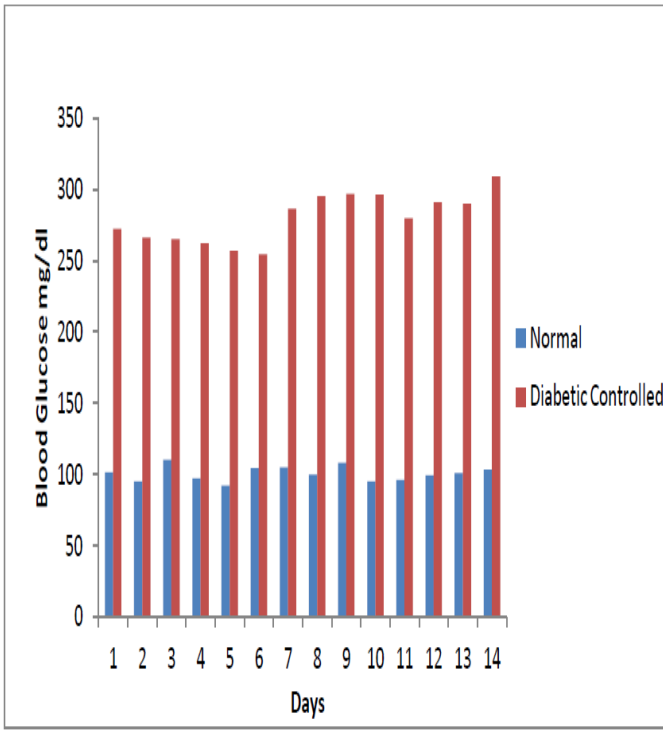

Figure 1. Evaluation of Blood glucose of normal and diabetic group.

N. oleander Shows Hypoglycemic Activity*:*

To evaluate the hypoglycemic activity of extract of *N. oleander* flowers, theblood glucose level were measured. The result showed the blood glucose level oftreatment group 1(150.64 ± 8.69) and treatment group 2 (118.66 ± 7.38) weremarkedly lower than diabetic group (308± 7.75) at day 14 as shown in Figure 4.2

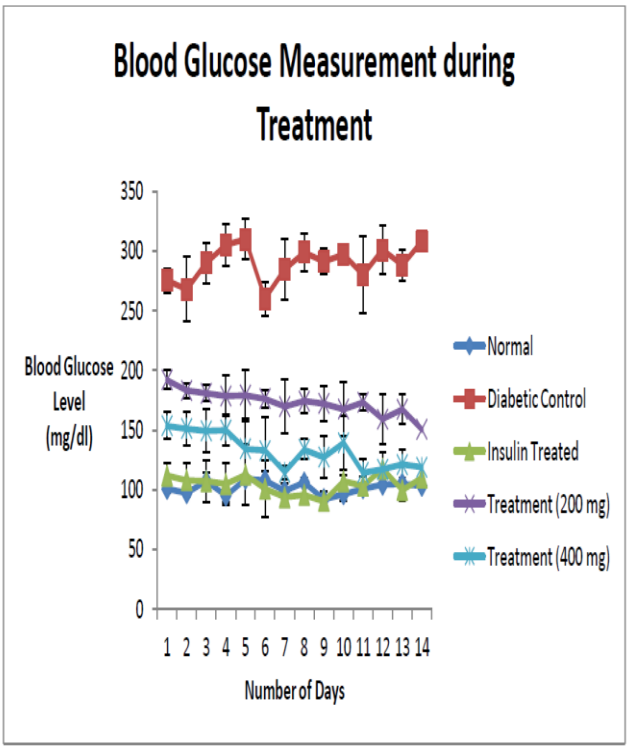

Figure 2. A Comparison of blood glucose levels of Normal, Controlled and Treatmentgroups

4.3: *Oral Glucose Tolerance Test:*

To check the impairment in glucose homeostasis, overnight fasted rabbitswere given glucose solution @ 3g/kg body weight. The blood glucose levels weremeasured at regular intervals of 30min for four times wise 30, 60, 90 and 120mins.The

result showed a significant decrease in blood glucose levels of treatment group1 (271.53 ± 10.5), (248.83 ± 9.87), (188.22 ± 8.29) and (170.22 ± 7.38) andtreatment group 2 (210.21 ± 18.45), (180 ± 17.9), (153.15 ± 14.25) and (151 ±13) as compared to diabetic group (328 ± 25.35), (288.52 ± 13.42), (272.42 ±9.88) and (255 ± 12) at 30, 60, 90 and 120mins respectively as shown in Figure 4.3

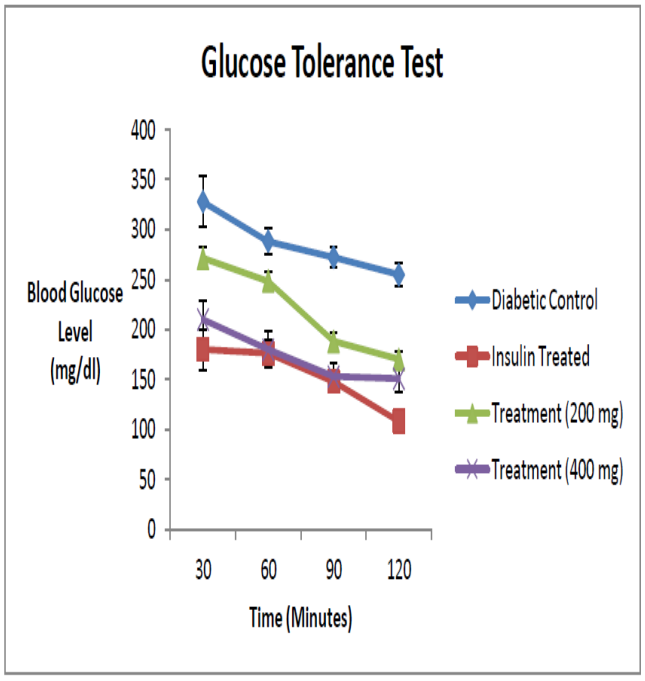

Figure 3. A Comparison of blood glucose of normal, control diabetic and treated groups. Allvalues are expressed in mean ± SEM.

4.4: Measurement of Serum Insulin Levels*:*

To assess the functionality of treated rabbits, the serum insulin levels weremeasured at the end of treatment. The results showed that there was a slight ornon-significant increase in the serum insulin level of treatment group 1 (5.67 ±0.18) and treatment group 2 (5.39 ± 0.9) as compared to diabetic group (3.73 ±0.256) as shown in Figure 4.4

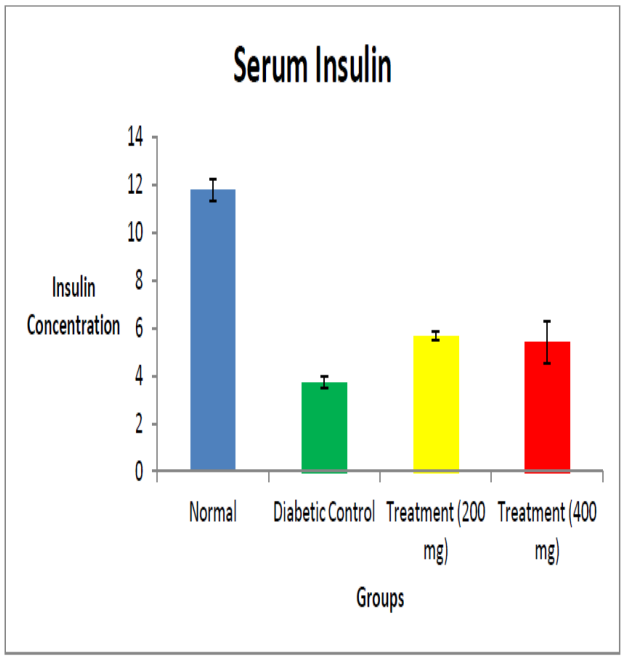

Figure 4. Serum Insulin Measurements (IU/ml) of Normal, Diabetic Control and Treatedanimals by ELISA. All values are expressed in mean ± SEM.

Histopathological Studies*:*

To observe the changes in morphology of pancreas after *N. oleander* flowersextract treatment, pancreata of rabbits from different groups were excised,processed, sectioned and stand with hematoxylin and eosin. The resultant standsections were observed under digital microscope. Images were taken and observedthe result showed slight improvement in the morphology of treatment groups ascompared to diabetic groups as shown in Figure 4.5

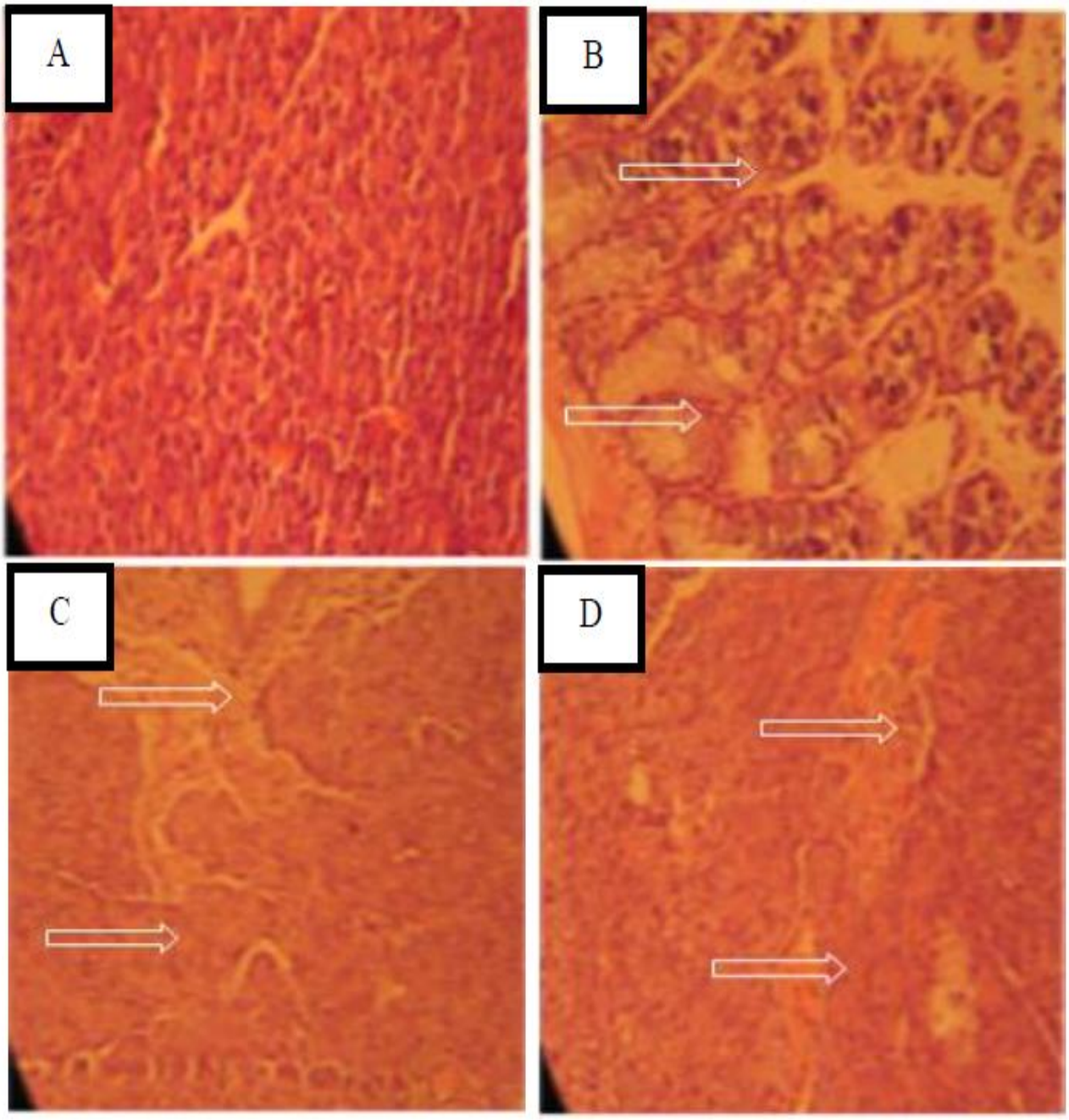

Figure 5. Histopathological micrographs of pancreatic section. Image A and B of Diabetic Control, C Treated with 200 mg/kg and D treated with 400 mg/kg

Conclusion:

After completion of present research it can be concluded that *NeriumOleander* is very effective against diabetes. The extract of flowers reduces theblood glucose level and also improved serum insulin levels. Histopathology ofpancreas also showed that there were significant changes after using the extract of*Nerium oleander*. Diabetes is a worldwide disease and the number of patients isincreasing rapidly. Although allopathic medicines are being used for treatment butit is common observation that these medicines have many side effects. So it ishighly recommended that phytomedicines should be preferred instead of allopathicmedicines and insulin injections. However the factor which limits the use of herbalmedicine is their standardization at commercial level.

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