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ANTI-DIABETIC EFFECT OF HYDROALCOHOLIC EXTRACTOF *NYMPHAEA PUBESCENS* ENTIRE PLANT IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

The present study explores the anti-diabetic activity of a hydroalcoholic extract of the entire *Nymphaea pubescens* (NP) plant in streptozotocin (STZ)-induced diabetic rats. STZ-induced diabetic Wistar rats had to take Metformin (10 mg/kg body weight) and NP hydroalcoholic extract (250 and 500 mg/kg body weight) orally for 21 days. Further, changes in the blood glucose level (BGL), body weight (BW), and biochemical parameters such as total protein (TP), serum creatinine (SC), serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamate pyruvate transaminase (SGOT) had observed. Pancreatic histology revealed that β-cells regeneration in islets of Langerhans.

KEYWORDS: *Nymphaea pubescens*, Anti-diabetic activity, Streptozotocin, and Metformin.

1. INTRODUCTION

Management of diabetes mellitus (DM) is a global problem, and successful treatment is needed to prevent or at least delay long-term issues like diabetic neuropathy, nephropathy, and retinopathy.[1] When treating these long-term conditions, herbal medicines or drugs with few side effects are better than synthetic drugs.[2] Even though doctors and researchers are still trying to find a complete and permanent cure for diabetes, herbal drugs as therapeutic agents are a natural boon compared to the severe side effects of allopathic medicine. It is thought that the traditional medications used to treat diabetes consider how the disease gets worse over time.[3]

Nymphaea pubescens have anti-diabetic activity. The literature review shows no anti-diabetic activity has been reported on this plant. Hence, this study has explored the anti-diabetic potential of *Nymphaea pubescens* on streptozotocin-induced diabetes in Wistar rats.[4]

2. MATERIALS AND METHODS

2.1. Plant material collection

The complete *Nymphaea pubescent* plant was collected from different locations in Andhra Pradesh. Dr. K. Madhava Chetty, an assistant professor in the botany department at Sri Venkateshwara University in Tirupati, confirmed that the plant specimen was confirmed.

2.2 Extraction of plant material

The sample was washed with distilled water to remove bits of glue, then dried in the shade and ground into a powder. The sample (20g) was weighed and extracted with a hydroalcoholic (25:75) solution bycontinuous hot percolation with the help of Soxhlet's apparatus for 12hrs. On completion, the extract became filtrated and concentrated using a rotary evaporator under reduced pressure and a temperature of 40 to 55^{0}C. The concentrates went into storage until further use.[5]

2.3. Experimental animals

Wistar rats (200–230 gm) were selected for the experimental study. The animals were procured from the animal house of Sainath Agencies, Hyderabad. The animals were kept and maintained under laboratory conditions of temperature (21.5 ± 22°C), humidity (60 ± 1%), and a 12-hour light/dark cycle. They were allowed free access to food (standard pellets) and water *ad libitum*. The IAEC of CPCSEA provided ethical committee clearance (IAEC/08/CCPER/CPCSEA2022).

2.4. Induction of diabetes

Diabetes was induced by injecting freshly prepared streptozotocin (STZ) at 50 mg/kg i.p, in 0.1 M cold citrate buffer, pH 4.5. The animals are investigated after 48 hours for glucosuria and hyperglycemia, with fasting blood glucose levels (BGL) more than 200 mg/dL. They were considered diabetic and used for further study.[6]

2.5. Experimental design

The diabetic Wistar rats were divided into a group of six

rats each and treated as follows: Group I were normal rats that received a standard saline solution, and Group II were STZ (50 mg/kg b.w., i.p) induced diabetic rats that served as a diabetic control group. Group III STZ (50 mg/kg b.w., i.p) induced diabetic rats were treated with Metformin (10mg/kg b.w/p.o). Group IV STZ (50 mg/kg b.w., i.p) induced diabetic rats were treated with hydroalcoholic extract of NP 250mg/kg b.w/ p.o. Group V STZ (50 mg/kg b.w., i.p) induced diabetic rats were treated with hydroalcoholic extract of NP 500mg/kg b.w/ p.o for 21 days.[7]

2.6. Determination of blood glucose level (BGL)

The BGL has measured before the administration of the extracts. On the first, seventh, fourteenth, and twenty-first days of treatment, the BGL was measured. The rat's tail was severed to gather blood. A glucometer is utilized to determine BGL (One Touch).

2.7. Estimation of biochemical parameters

The blood samples were collected, and serum was separated using a centrifuge to study the biochemical parameters. The estimation of protein took place using the method of Lowry. The SGOT and SGPT have been measured by the process of Reitman and Frankel (Colorimetric method). Jaffe's method evolved to calculate serum creatinine.[8]

2.8. Histopathological study

The animals seemed then slaughtered while under mild ether anesthesia. The rats were sacrificed by decapitation, and relevant organs like the pancreas were removed, dissected, and washed with ice-cold saline. For histological examinations, the organs remained in a 10% formalin solution.[9]

2.9. Statistical analysis

The data were expressed as mean ± standard error (SEM). The significance of differences among the groups was assessed using a one-way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered significant.[10]

3. RESULTS AND DISCUSSION

The hydroalcoholic extract from the whole plant of *Nymphaea Pubescens* was subjected to preliminary phytochemical analysis, which showed the presence of flavonoids, alkaloids, tannins, proteins, steroids, and phenol with an absence of saponins and anthraquinone. Flavonoids are also known to regenerate the damaged *β*-cells in diabetic mice.

The effects of Metformin and hydroalcoholic extracts on BGL after treatment of 21 days. In which extracts showed significant reduction ($p<0.01$). It observed that the standard drug metformin lowered the blood glucose levels significantly, bringing them back to normal, indicating the presence of some *β*-cells. In STZ-induced diabetic rats, there was a substantial decrease in average weight. Diabetes weight loss has caused by continuous glucose excretion and reduced peripheral glucose absorption and glycogen synthesis. Fig 1 depicts the BGL and body weight findings.

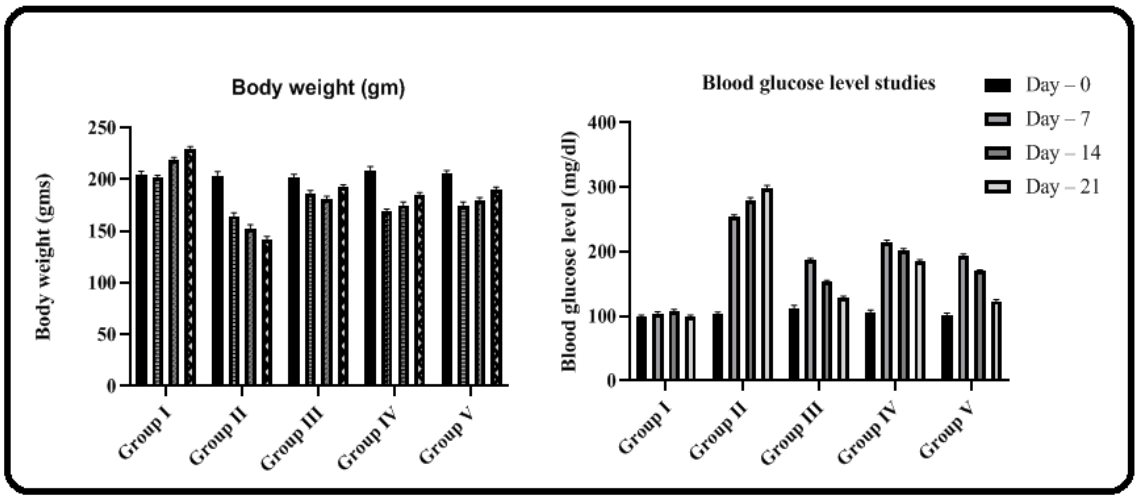


Fig 1: Effect of hydroalcoholic extract of NP on body weight and blood glucose levels.

Diabetic hyperglycemia induces elevation of the serum levels of creatinine, which are significant markers of renal dysfunction. The treatment of hydroalcoholic extract of NP in rats showed a marked decrease in serum creatinine levels and total protein content in diabetic animals; Fig 2 illustrates the results.

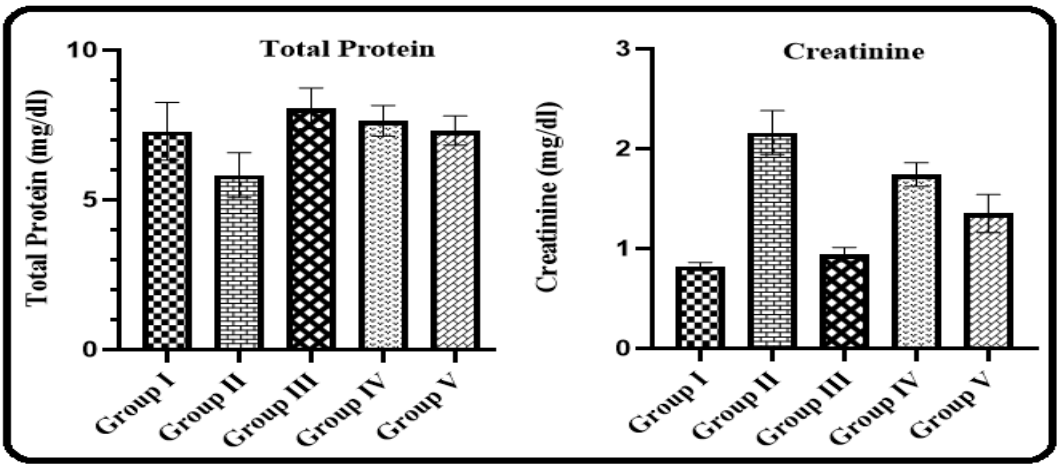


Fig 2: Effect of hydroalcoholic extract of NP on total protein and creatinine.

In SGOT and SGPT, enzyme levels get elevated during liver damage, which is higher in diabetic rats. The diabetic rats treated with Metformin and hydroalcoholic extract NP reduced the SGOT and SGPT levels; the results are displayed in Fig 3.

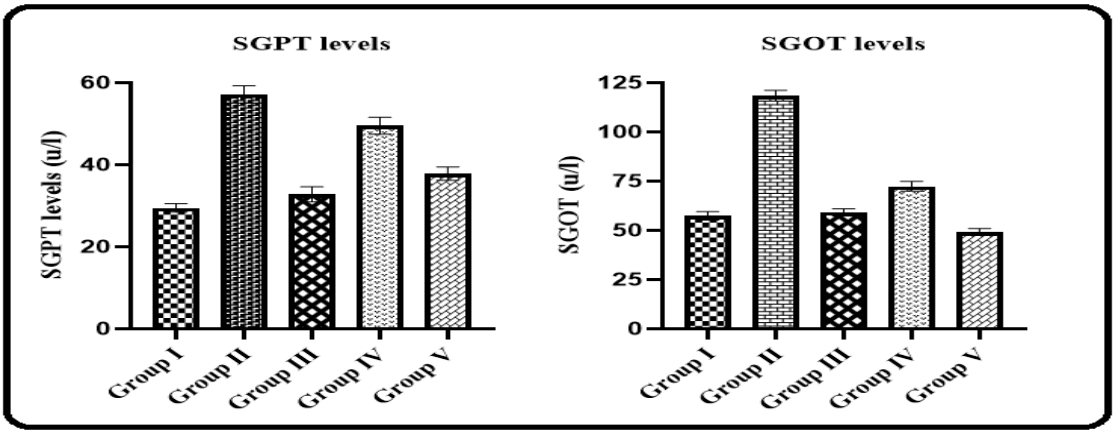
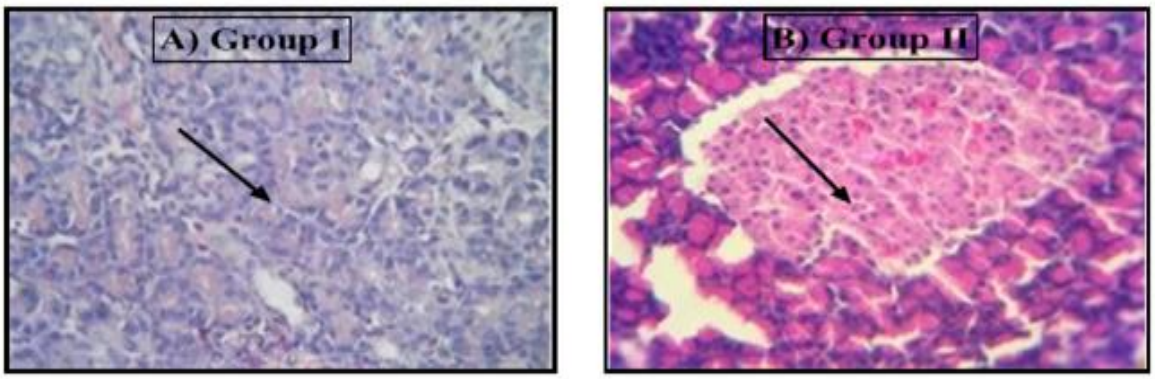


Fig 3: Effect of hydroalcoholic extract of NP on SGPT and SGOT levels.

The hydroalcoholic extract of NP-treated diabetic rats and pancreas histology revealed normal islets in the pancreas with standard structure compared to normal rats, possibly due to the anti-diabetic efficacy. It illustrates Group I normal islets of Langerhans with granulated cytoplasm in normal Wistar rats. Group II, Irregular necrotic pancreatic and cytoplasmic degeneration were observed in STZ-induced Wistar rats. In the case of Group III (STZ and Metformin), Group IV & V with hydroalcoholic extract of NP (250 and 500 mg/kg body weight) treated shows restoration in the islets of Langerhans were observed; the results presented in Fig 4.



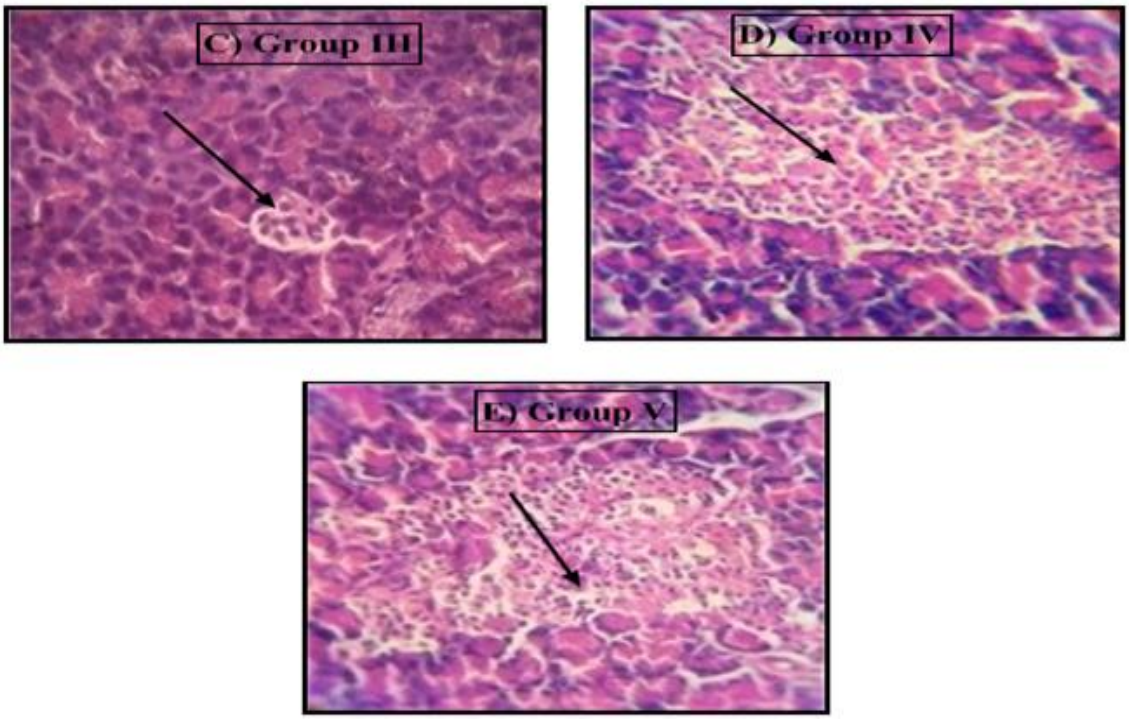


Fig 4: Histopathology analysis of pancreas (Magnification HE. 40x).

4. CONCLUSION

The anti-diabetic activity of the hydroalcoholic extract of the NP entire plant is shown by body weight, blood glucose level, estimation of total protein content, and the apparent reduction in SGOT, SGPT in the liver, and creatinine in serum. The NP also reduced pancreas damage. Thus, it may be concluded that NP observed a significant anti-diabetic activity in STZ-induced diabetic Wistar rats. The efficacy of the NP was comparable to that of Metformin. However, further studies are necessary to isolate bioactive compounds from NP; this could be a limitation of the study providing a new strategy for managing diabetes.

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