

ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF BLECHNUM SP. EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RAT MODELS

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ABSTRACT

In this study we investigate the therapeutic potential of *Blechnum* sp. plant extract in Streptozotocin (STZ) induced diabetic rat models. To establish the efficacy of *Blechnum* sp. we performed the biochemical studies including examine the blood glucose, cholesterol, and HDL levels. We found that our selected plant significantly reduced blood glucose and cholesterol while partially restoring HDL, which demonstrating their protective role in STZ induced diabetes. In addition, we also performed the Histopathological studies to comprehend the role of *Blechnum* sp in effectively restoring the degeneration and necrosis of liver tissues. It also revealed that plant extract treatment improved hepatocyte architecture and reduced pathological changes. Further, to validate the results we also examine the gene expression by using RT-PCR. Which exhibits the stringent agumentation of TNF- α and IL-6 with down regulation of IL-10 in STZ induced diabetic model, confirming a pro-inflammatory condition. Although, after the treatment of *Blechnum* sp. it attenuates the TNF- α and IL-6 expression and improved IL-10 levels, suggesting its immunomodulatory action. Whereas marketed drug Metformin exert to normalize the effect produced by STZ. Moreover our finding suggests the potential antidiabetic efficacy of *Blechnum* sp. And may in near future used for diabetes management.

Keywords: Diabetes mellitus; *Blechnum occidentale*; Antidiabetic; Antioxidant; Streptozotocin.

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Introduction

Diabetes mellitus (DM) is considered as one of the five fatal leading causes of death in the world (Pan C et al 2025). In a recent report published by WHO that about 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates ten years ago and this may double by the year 2030 (Hossain MJ et al 2024). Although in India the diabetes patients are increasing day by day which ultimately leads the more population are affected (Pradeepa R et al 2021). Based on reported literature the occurrence of DM is expected to rise more rapidly in the coming future because of augmenting inactivity and obesity (Khan MAB et al 2020).

Moreover natural products are safer and effective for DM (Pan C et al 2025). As a result, more and more drugs are coming up

offering newer and better options for the treatment of DM, but their critical clinical evaluation has recorded several discrepancies including drug interactions and adverse side effects (Olanrewaju OA et al 2023). So if we develop drugs from native sources are thus now a novel target for anti-diabetic treatment. Moreover, Indian medicinal plants possess enormous potential to manage and cure the diabetes (Gaonkar VP et al 2020).

In our literature survey we found that the *Blechnum occidentale* plant and their parts like leaves, and other isolated compounds possessed therapeutic properties against different diseases (Lai HY et al 2010). *Blechnum* showed efficacy against blisters, influenza, and intestinal parasite like worms, pulmonary complications, typhoid fever, urinary bladder diseases, and fatal wounds. Additionally our selected plant is rich in active

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phyto-ingredients like fatty acids, phenolic compounds, lipids and sterols (Andrade JM et al 2016). Further *Blechnum occidentale* plant has been used as anticancer, antimicrobial, insecticidal, anti-inflammatory, antioxidant (Nonato FR et al 2009), anti-trematocidal and significant potential to heal the detrimental wounds (Waswa, E.N et al 2022). In addition, leaves of *Blechnum* used to treat lungs complications, stomach and their precious form cancer. Therefore, investigation on *Blechnum* has become more important. Hence in this study we established the anti-diabetic activity of *Blechnum occidentale* which has not been reported earlier.

Detailed methodology

Chemicals used in the present study have been procured from sigma and SRL.

Plants material

Blechnum occidentale plants were collected and the voucher specimen numbers were assigned and preserved in the Herbarium of the Institute.

Experimental Animals

Experimental protocols were approved by the Institutional Ethical and Usage Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy). Adult male Sprague Dawley rats, weighing 160–230 g were used in this study. Animals were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms with temperature regulated at $22 \pm 2^\circ\text{C}$, 12/12 h light and dark cycle.

Treatment schedule

Pilot investigation with the graded doses of test compounds were tested against STZ model to identify the effective dose and selected for further studies. Various test compounds; reference drug Metformin (MET) (10 mg/kg) were freshly prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally to the animals. The rats were randomly divided into various groups, each consisting of 6 animals.

Treatment groups for diabetic model

Rats were divided into three groups.

Group I (Control group): Control group of animals were treated with 1% CMC, for 21 d after induction of diabetes in rats.

Group II (Treatment groups): Rats were treated with *Blechnum occidentale* herbal extract. Rats administered with STZ (45 mg/kg body weight) intraperitoneally. Oral administration of plant extract *Blechnum occidentale* (200 mg/kg body weight) in STZ induced rats from Day 8 to Day 21.

Group III (Reference drug treated): Rats were treated with marketed drug metformin (100mg/kg) after 72 hr of streptozotocin treatment.

Streptozotocin induced diabetes models

Male Sprague Dawley strain albino rats of average body weight $160 \pm 230\text{gm}$ having blood glucose profiles between 60-80 mg/dl were selected. To develop the diabetes Streptozotocin (STZ) (Sigma, USA) was administered. STZ was dissolved in 100 mM citrate buffer (pH 4.5) and calculated amount of freshly prepared solution of streptozotocin was injected intraperitoneally (i.p.) to overnight fasted rats at a single dose of 45 mg/kg body weight (Ghasemi A et al 2023). The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia. After 72 h of STZ administration, blood samples were collected from tail and glucose levels were estimated by glucostrips (One Touch Glucometer, Life Scan, Europe). Animals having fasting blood glucose levels above 200 mg/dl were considered diabetic and subsequently used in the present study.

Dose selection

To decide the effective dose we administered the *Blechnum occidentale* at different doses to SD rats (100, 200, and 400 mg/kg body weight) after 72 h of STZ (Sigma, USA) was administered intraperitoneally (i.p.) to overnight fasted rats (45 mg/kg body weight). After herbal extract administration, blood samples were collected from tail and glucose levels were estimated by glucostrips (One Touch Glucometer, Life Scan, Europe).

Body Weight Measurement

For body weight evaluation, all experimental animals were weighted at the initiation of the experimental duration and after five weeks. The body weights were recorded at recording time in the morning according to Al-Attar et al

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2010. Furthermore, for any signs of abnormalities throughout the duration of investigation, the rats were continuously observed.

Biochemical estimation in streptozotocin induced diabetic models

Preparations of serum, plasma and tissue homogenate after the experimental period, animals were scarified by cervical decapitation. Blood was collected and centrifuged for serum separation. For plasma, blood was collected with anticoagulant and centrifuged at 2000 r/min for 20 min. The resulting supernatant was used for the estimation of blood glucose (Aku Enam Motto et al 2022) cholesterol, high density lipoprotein (HDL) and triglyceride (Almalki DA, et al 2019).

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay

Antioxidant activity of *Blechnum* sp. extract was evaluated using the DPPH assay. A 0.1 mM DPPH solution in methanol was mixed with varying concentrations of extract, incubated for 30 minutes in the dark, and absorbance measured at 517 nm. Radical scavenging percentage was calculated against control, with ascorbic acid as standard.

Scavenging/Inhibitory activity coefficient: The scavenging or percentage inhibitory activity of in each assay was calculated from:

$$\% \text{ Inhibition} = (A_0 - A_1) \times 100 / A_0$$

Where A_0 = absorbance of the control (without test compounds), and A_1 = absorbance of the treated (with test compounds). The IC_{50} value was extrapolated from the standard linear regression curve.

Histo-pathological studies

Both control and experimental rats were killed by decapitation and liver tissues were isolated. Liver tissue were dipped into 4% formalin for 24 hr. to stain the liver tissue cut into thin section and kept it on micro tech slides and fixed. Liver tissues were leave for 24h and observed under microscope (10X).

Expression of pro (TNF-alpha & IL-6) and anti-inflammatory cytokines (IL-10) via RT-PCR.

Total RNA was isolated from liver tissues of control, STZ-diabetic, and treated rats. cDNA was synthesized and amplified using specific primers for TNF- α , IL-6, and IL-10. PCR

products were analyzed on agarose gels, normalized to β -actin, and relative expression calculated.

Statistical analysis:

Data were expressed as mean \pm S.E.M. Analysis will be performed with Prism version 5.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered to be statistically significant.

Results & Discussion

Glucose level

After 72 hr of STZ induced hypoglycemia analysis blood glucose profile was checked by glucostrips (Boehringer Mannheim). Animals were showing blood glucose values after 24 48 and 72 hr 60, 100, 320 mg/dl were included in the experiments and termed diabetic. To cure the diabetes we administered animals with different doses of *Blechnum* (100, 200, and 400 mg/kg body weight). The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia. Animals having fasting blood glucose levels above 200 mg/dl were considered diabetic and subsequently used in the present study.

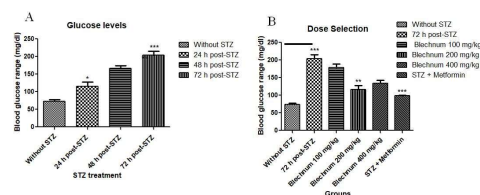


Figure: In image A Blood glucose profile was checked for 3 consecutive days (i.e. 72 hours) after STZ treatment by glucostrips (Boehringer Mannheim) whereas in graph B it depicts the effective dose of *Blechnum* significantly reduce the blood glucose values between 130 to 145 mg/dl at 200 mg/kg body weight. Whereas the reference drug metformin showing prominent effect on STZ induced diabetes model.

Biochemical Analysis

In biochemical analysis, control animals showed low blood glucose, total cholesterol, high HDL and triglyceride, 86.67mg/dl, 87.33mg/dl, 42.67 and 79.00 respectively reflecting balanced metabolism. Whereas STZ-diabetic rats exhibited marked hyperglycemia, elevated cholesterol, and reduced HDL, indicating severe metabolic

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disturbance. Treatment with *Blechnum sp.* extract significantly lowered glucose as well as cholesterol, while partially restoring HDL, and lowering triglyceride suggesting protective effects. Whereas reference drug Metformin treated animals demonstrated the reversion of STZ induced effect which confirming their prominent efficacy. Overall, our results highlight that plant extract *Blechnum* have potential to improves the metabolic disorders.

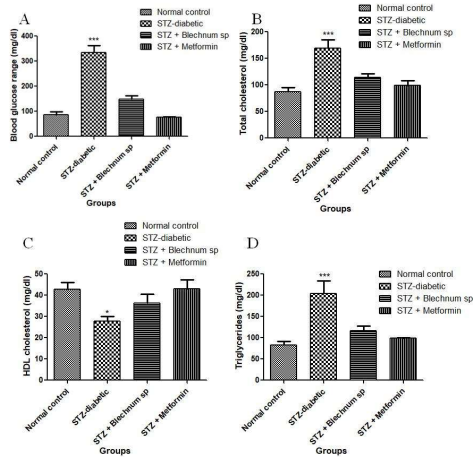


Figure: Effect of *Blechnum* and reference drug Metformin (Met) on STZ induced diabetic models in rats. Data expressed as mean of % protection of CA and reference drugs after diabetes induction \pm S.E.M. Statistical analysis was done by one way ANOVA followed by Dunnett’s Multiple Comparison Test. *Statistically significant at $p < 0.05$ and ** $p < 0.01$, in comparison to control. $n = 6$ in each group.

Antioxidant activity of Bichenum by using DPPH

DPPH activity was performed by following the Viturro et al 1999 based on the free radical scavenging activity. *In vitro* antioxidant lowers the load of ROS and ultimately which impact the stress related disorders. *In vitro* antioxidant studies of *Blechnum* extract was performed to understand the antioxidant activity of *Blechnum*. Whereas our result in Table 1 demonstrated that our plant extract was effective in scavenging (Baliyan S et al 2022).

SN.	Concentration (µg/ml)	1,1-Diphenyl 1-2-picryl-
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		hydrazyl (DPPH)
1	10	18.39 \pm 2.54
2	20	31.70 \pm 4.81
3	40	44.06 \pm 9.15
4	80	92.00 \pm 23.87
5	160	112.58 \pm 27.46
6	IC 50	85.62 \pm 14.93
7	Reference (ascorbic acid) IC50	43.72 \pm 4.01

Table1: Represents antioxidant activity analysed by the DPPH assay.

Histo pathology of liver

Figure 3 section A is the image of liver of normal rat, depicting the intact hepatocyte cords and ubiquitous staining indicates the normal condition of liver. This also set the baseline to compare with other treatment sections. In panel B photograph of STZ induced diabetic liver indicating the fat vacuoles in hepatocytes, disrupted parenchymal architecture, all of them represents pathological damage due to STZ induced diabetes. Whereas in panel C highlighted the improvement after the treatment of our plant extract *Blechnum*. It demonstrates the decreasead numbers of vaccoules, tight arraengement of liver cells and also it seems that restoration of their integrety. This all represents the potential of our plant extract against STZ induced diabetes. Here panel D highlights the effect of marketed drug; we used Metformin in our study as reference, on re-establishment of liver cells. Metformin reduces the inflammation and fat accumulation. Also we used panel D to compare our plant efficacy against diabetes.

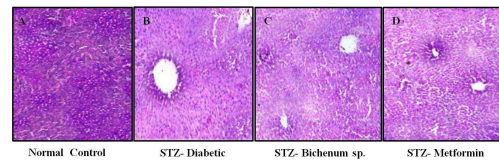


Figure 3. Staining image of liver regions of Control, STZ-groups, *Blechnum* treated groups and reference drug metformin that exert to revert the STZ effect.

RT-PCR analysis

RT-PCR expression data exhibited the low expression of pro-inflammatory cytokines like

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TNF- α & IL-6 and the high expression of anti-inflammatory cytokine IL-10 in normal rats. Whereas in STZ-induced diabetic rats the stringent upregulation of TNF- α & IL-6 and marked suppression of anti-inflammatory cytokine IL-10. When we compared these results with *Blechnum sp. extract* reduced expression of TNF- α & IL-6 and while partially restoring the expression of anti-inflammatory state IL-10. Whereas reference drugs Metformin exhibited prominent activity against diabetes and exerts the near normal conditions of cytokine profiles, further confirming their immunomodulatory effects. Hence, in our study we found that *Blechnum* plant extract have potent anti diabetic activities.

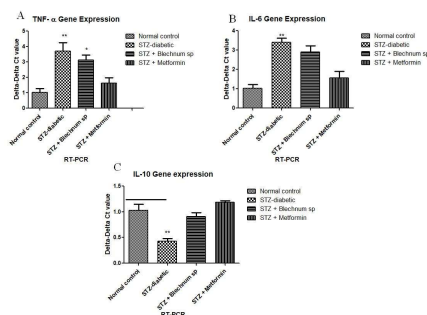


Figure 2: Shows RT-PCR gel bands for TNF- α , IL-6, and IL-10 expression.

Conclusion

Identifying research gaps in diabetes is crucial for directing future research efforts effectively and addressing areas where knowledge is lacking. Research is needed to better understand the mechanisms underlying the development of type 1 diabetes and to identify potential interventions for prevention. There are significant disparities in the prevalence, management, and outcomes of diabetes among different populations, including racial and ethnic minorities, socioeconomically disadvantaged groups, and rural communities. Furthermore, this includes evaluating the cost-effectiveness of different treatment modalities, screening strategies, and lifestyle interventions, as well as the impact of health policies and reimbursement systems on diabetes care delivery. By addressing these research gaps, scientists and healthcare professionals can work towards improving the prevention, management, and outcomes of diabetes and ultimately reducing the global burden of this chronic disease.

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