Evaluation of the Antidiabetic Activity of Northeast Indian variety of *Zingiber rubens* and Exploring the Mechanism of Action

ABSTRACT

Submitted By

Sujit Das, M.Pharm.

Reg. No. 1021813005

Doctor of Philosophy (Pharmacy)

Division of Pharmacology Department of Pharmaceutical Technology Faculty Council of Engineering & Technology

Jadavpur University Kolkata,

India

2024

Diabetes is becoming more common due to urbanization and poor lifestyle choices, significantly affecting the health and economy of developing nations. In India, the International Diabetes Federation (IDF) projected an increase from 50.8 million diabetics in 2010 to 87 million by 2030, with rising prevalence in both urban and rural areas. Current oral hypoglycaemic medications have side effects like heart failure and gastrointestinal issues, underscoring the need for safer treatments. Multi-target therapeutic agents are essential to tackle the complexities of Type 2 diabetes (T2DM), and plant extracts are being investigated for their potential in diabetes management. Medicinal plants significantly manage and treat diabetes, offering valuable alternatives and complementing conventional therapies.

Several Indian medicinal plants are noted for their antidiabetic properties, including *Zingiber rubens* [Family Zingiberaceae], widely used. The antidiabetic properties of this plant have yet to be confirmed through rigorous experimental studies, and the specific molecular mechanisms underlying its potential antidiabetic effects remain unexplored. Further research is needed to investigate how this plant may contribute to blood sugar regulation and identify the biochemical pathways involved in its activity. Thus, the objectives of the study was to validate the anti-diabetic activity of *Zingiber rubens* in experimental models and to explore the underlying mechanism of action for the anti-diabetic activity.

In this context, a series of studies were meticulously planned and executed, beginning with an investigation into the in vitro free radical scavenging activity as well as the potential antidiabetic effects of the hydroalcoholic root extract of the plant (referred to as HAZR). Following the initial findings, the study ventured into an in vivo evaluation of HAZR's antidiabetic properties, aiming to uncover the underlying molecular mechanisms by which the extract modulates oxidative stress, impacting various tissue biochemical parameters and levels of HbA1c.

To gain a deeper understanding of these processes, an assay was conducted to assess the inhibition of protein kinase C (PKC) phosphorylation in mouse peritoneal macrophages. This was essential for elucidating a comprehensive molecular mechanism related to the extract's effects. Additionally, preliminary predictions regarding the association between genes and diseases were carried out using network pharmacological assays, providing further insights into the potential therapeutic implications of HAZR.

Fresh rhizomes and roots of *Zingiber rubens* were collected and dried to preserve their properties. They were then ground into small fragments and sifted through a 100-micrometer mesh for uniform particle size. The crude powders were packed into a thimble and placed in a Soxhlet apparatus filled with a 70% methanol and 30% water mixture. The percentage yield was 11.25% (W/W). The antioxidant potential of HAZR was assessed using the DPPH (2,2diphenyl-1-picrylhydrazyl) radical scavenging assay, with ascorbic acid used as the reference standard. The results, illustrated in Figure 1, show the percentage inhibition of DPPH activity at different concentrations of the extract. HAZR displayed significant effectiveness in scavenging free radicals, with an IC50 value of 25 µg/ml. In comparison, the standard ascorbic acid had an IC_{50} value of approximately 42 µg/ml. The inhibitory effect of HAZR on α-glucosidase was evaluated by calculating its IC_{50} value at various concentrations, using Acarbose as the standard inhibitor. HAZR's IC_{50} was found to be 345.6 ± 5.63 µg/ml, compared to Acarbose's significantly lower IC_{50} of 51.2 ± 1.63 µg/ml, indicating HAZR's lesser effectiveness.

The analysis of seven selected compounds focused on their interactions within a network of 297 targeted proteins and enzymes, visualized using CYTOSCAPE. The 7 chosen compounds were represented by solid lines, showcasing their interconnections. Compounds with the highest interaction degrees were highlighted with a red dotted circle. Most isolated compounds primarily interacted with three key proteins: the 5′-AMP-activated protein kinase catalytic subunit alpha-2/β 1/γ 1, 1,3-beta-D-glucan synthase, and xanthine dehydrogenase/oxidase.

In the *in vivo* study, male Wistar albino rats weighing 160 to 180 grams were supplied by M/S Chakraborty Enterprise in Kolkata (Reg. No. 1443/PO/Bt/s/11/CPCSEA). Prior to the experimental procedures, the rats had fasting blood glucose levels of 85 to 90 mg/dL. They underwent a 7-day acclimatization period with unlimited food and water. All experimental protocols were reviewed and approved by the Animal Ethical Committee at Jadavpur University (Approval No. JU/IAEC-22/47) on June 15th, 2023, ensuring ethical compliance.

Rats with normal blood sugar levels (85 to 90 mg/dl) underwent an oral glucose tolerance test to assess their metabolic response. After glucose administration, blood sugar levels spiked sharply within the first 30 minutes, followed by a gradual decline over the next 90 minutes, indicating the body's glucose regulation mechanisms. Notably, a dosage of 400 mg/kg body

weight of HAZR significantly reduced blood glucose levels in the rats during the test, suggesting a potential therapeutic effect of HAZR on glucose metabolism. A study on diabetic rats induced by low-dose streptozotocin (STZ) and a high-fat diet showed that these animals had significantly higher fasting blood glucose (FBG) levels compared to normal controls ($p < 0.05$). Diabetic rats were treated with HAZR for 14 days at doses of 200 mg/kg and 400 mg/kg, resulting in a significant reduction in FBG levels and bringing them closer to normal ($p < 0.05$). Additionally, HAZR treatment led to a significant decrease in glycosylated hemoglobin levels compared to untreated diabetic rats ($p < 0.05$), as shown in Figure 2. These findings suggest that HAZR may help manage glucose levels in diabetes.

Compared to the control group on a low-dose STZ high-fat diet, groups treated with HAZR showed a significant reduction ($p<0.05$) in key blood markers, including SALP, SGOT, and SGPT, suggesting improved liver function. Additionally, treated groups had increased total protein levels compared to controls, indicating enhanced protein synthesis. These findings suggest that HAZR has hepatoprotective properties, effective at both low and high doses. A study comparing HAZR-treated rats to diabetic rats revealed significant biochemical changes. The level of malondialdehyde (MDA), a marker of oxidative stress, was significantly reduced in the HAZR-treated group ($p<0.05$), suggesting protection against cellular damage. Additionally, levels of superoxide dismutase (SOD) and glutathione (GSH), key antioxidants, were significantly elevated ($p<0.05$). These results indicate that HAZR treatment not only reduces oxidative stress but also boosts the body's antioxidant defenses. Diabetic rats displayed significant changes in their serum lipid profiles compared to normal rats. They had elevated levels of triglycerides and total cholesterol ($p < 0.05$), while HDL (high-density lipoprotein) levels were significantly lower ($p < 0.05$).

Moreover, histopathological examinations of pancreatic tissue revealed compelling evidence of HAZR's protective effects on pancreatic beta cells, which are crucial for insulin production. Remarkably, when comparing the treated groups to diabetic rats, there was a clear, dose-dependent improvement in the density of these vital beta cells. This gradual enhancement underscores the beneficial impact of HAZR treatment on pancreatic health, as depicted in Figure 6. Overall, these findings highlight the potential of HAZR to mitigate the adverse effects of diabetes on pancreatic structure and function.

In this investigation of the molecular mechanisms underlying the action of HAZR, we focused on its effect on the phosphorylation of protein kinase C (PKC) in mouse peritoneal macrophage cells. PKC plays a crucial role in the phosphorylation process of AMP-activated protein kinase (AMPK), which is vital for endocrine regulation. These findings revealed that HAZR significantly suppresses the phosphorylation of PKC, particularly in response to stimulation with lipopolysaccharide (LPS). This is clearly illustrated by the observed gradual increase in PKC levels at various escalating concentrations of the plant extract. Conversely, we noted a decrease in the levels of phosphorylated PKC (p-PKC), which highlights the inhibitory action of HAZR on the phosphorylation of PKC.

Protein kinase C (PKC) is essential for the phosphorylation of AMP-activated protein kinase (AMPK), which regulates endocrine functions. This research indicates that HAZR significantly inhibits PKC phosphorylation in cells exposed to lipopolysaccharide (LPS), an inflammatory agent. Both LPS and streptozotocin (STZ) equally induce inflammatory responses, complicating these interactions. This inhibition suggests that oxidative stress may affect PKC activity and TGF-β regulation, particularly in individuals with diabetes, enhancing the understanding of diabetic pathology.

This study shows that HAZR has a significant anti-diabetic effect in diabetic animal models, primarily through oxidative stress-mediated regulation of PKC and TGF-β, presenting promising therapeutic opportunities. Key conclusions include:

Signature of the Candidate Sujit Das 25/11/24.

Signature of the Supervisor(s)

1. Pallab Kanti Haldar 25/11/24

Prof. Pallab Kanti Haldar
M. Pharm., Ph.D., FIC
Division of Pharmacology & Toxicology
Dept. of Pharmaceutical Technology
Jadavpur University, Kolkata-700032

2. 25/11/2024

Dr. Manas Bhowmik
Associate Professor
Dept. of Pharmaceutical Technology
Jadavpur University, Kol-700 032
West Bengal, India