Investigation of Anti-Diabetic Activity of Ethanolic Leaves Extract of Dichrostachys cinereal

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KEYWORDS

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ABSTRACT

Diabetes mellitus is a significant global health challenge, necessitating innovative and complementary therapeutic approaches. The present study investigates the anti-diabetic potential of Dichrostachys cinerea, a plant with a rich history in traditional medicine. Despite anecdotal claims of its efficacy in diabetes management, scientific validation remains limited. This research aims to bridge this gap through a comprehensive exploration of the pharmacological properties of Dichrostachys cinerea. Fresh leaves of Dichrostachys cinerea were collected, authenticated, and subjected to ethanolic extraction. Phytochemical analysis confirmed the presence of key bioactive constituents such as phytosterols, flavonoids, carbohydrates, and terpenoids. To evaluate its anti-diabetic effects, Sprague-Dawley rats were divided into control and experimental groups, including diabetic and treated groups receiving Dichrostachys cinerea extract at doses of 250 mg/kg and 500 mg/kg for 21 days. The efficacy of the extract was assessed through fasting plasma glucose, serum insulin levels, glycosylated hemoglobin (HbA1C), lipid profiles, and histopathological examinations of liver and pancreas tissues. Results demonstrated a significant reduction in fasting plasma glucose levels, improved glucose tolerance, and enhanced serum insulin levels in the Dichrostachys cinerea-treated groups compared to the diabetic control. Additionally, a dose-dependent positive impact on body weight regulation was observed. Histopathological analysis revealed restoration of normal architecture in liver and pancreas tissues, suggesting protective and regenerative effects.

1. Introduction:

1.1 Background:

Diabetes mellitus stands as a formidable global health challenge, manifesting a surge in prevalence and posing a multifaceted threat to public health worldwide(1). The intricate interplay of genetic predispositions, lifestyle factors, and environmental influences has contributed to the escalating incidence of diabetes(2). As conventional treatments grapple with the complexities of this metabolic disorder, the search for innovative and complementary therapeutic avenues becomes imperative.

In the pursuit of such alternatives, the plant Dichrostachys cinerea emerges as a subject of heightened interest. With a history deeply rooted in traditional medicinal practices, Dichrostachys cinerea holds anecdotal promise as a potential remedy for diabetes(3). The wealth of indigenous knowledge surrounding its applications in diabetes management beckons scientific scrutiny, urging a closer examination of its pharmacological properties.

1.2 Rationale:

Despite the intriguing traditional claims, scientific exploration into the anti-diabetic potential of Dichrostachys cinerea has been notably scarce. This research seeks to bridge this gap through a comprehensive and systematic investigation. The dearth of scientific studies examining the efficacy of Dichrostachys cinerea in diabetes management has created a critical void in our understanding of its therapeutic potential(4).

The rationale behind this investigation is rooted in the urgency to unravel the mysteries of Dichrostachys cinerea's pharmacological activity. By delving into its impact on crucial aspects of diabetes, such as glucose metabolism and insulin regulation, we aim to shed light on its potential as a therapeutic agent. The outcomes of this study have the potential to not only validate or refute traditional claims but also contribute meaningful insights to the broader landscape of diabetes management.

As we navigate through this exploration, the multifaceted nature of Dichrostachys cinerea's influence on diabetes will be dissected, unraveling its potential as a source of novel therapeutic interventions. This research endeavor holds the promise of unveiling a natural remedy that may significantly contribute to the global effort to mitigate the impact of diabetes on public health.

2. Materials and Methods:

2.1 Plant Material:

2.1.1 Collection and Authentication:

Fresh leaves of Dichrostachys cinerea were meticulously collected from their natural habitat in Kolli Hills, Namakkal. The plant material was then subjected to a rigorous authentication process, ensuring the accurate identification of Dichrostachys cinerea. Expert botanists verified the plant's identity, and a voucher specimen was deposited for future reference.

2.1.2 Preparation of Ethanolic Leaves Extract:

The ethanolic leaves extract of Dichrostachys cinerea was meticulously prepared to retain the maximum bioactive constituents. Fresh leaves were thoroughly cleaned, shade-dried to remove excess moisture, and finely powdered. The powdered plant material was then subjected to continuous hot extraction using ethanol as the solvent. The resulting extract was carefully concentrated, yielding the ethanolic extract used for subsequent analyses.

2.2 Phytochemical Analysis:

The ethanolic leaves extract underwent a comprehensive phytochemical analysis to identify and quantify its chemical constituents. The following standard procedures were employed for the detection of specific phytochemical classes:

2.2.1 Phytosterols:

Test: Salkowski test

Observation: Development of a red color indicates the presence of phytosterols.

2.2.2 Flavonoids:

Test: Shinoda test

Observation: Intense color change signifies the presence of flavonoids.

2.2.3 Carbohydrates:

Test: Molisch's test

Observation: Formation of a violet ring indicates the presence of carbohydrates.

2.2.4 Terpenoids:

Test: Salkowski test

Observation: Development of a reddish-brown color indicates the presence of terpenoids.

The results of the phytochemical analysis provide a detailed profile of the chemical constituents present in the ethanolic leaves extract, forming the foundation for further investigations into its anti-diabetic potential.

2.2 Experimental Design:

2.2.1 Grouping of Rats:

Sprague-Dawley rats were systematically divided into distinct groups to facilitate a robust experimental design. The groups included:

1. Control Group: Rats administered only Carboxy Methyl Cellulose (CMC).
2. Diabetic Control Group: Rats induced with diabetes (STZ 65mg/kg + 110mg/kg Nicotinamide body weight) without treatment.
3. Glibenclamide Group: Diabetic rats treated with Glibenclamide (10 mg/kg p.o) for 21 days.
4. Dichrostachys Cinerea Extract (DCE) Groups:

a. Diabetic rats treated with Ethanolic Extract of Dichrostachys Cinerea (DCE) - 250 mg/kg p.o for 21 days.

b. Diabetic rats treated with Ethanolic Extract of Dichrostachys Cinerea (DCE) - 500 mg/kg p.o for 21 days.

2.2.2 Treatment Duration:

All groups underwent a 21-day treatment period, during which the rats received their designated treatments as outlined in the experimental design.

2.3 Biochemical Parameters:

2.3.1 Fasting Plasma Glucose:

Fasting plasma glucose levels were estimated using the glucose oxidase/peroxidase method. Blood samples collected from fasted rats after the 21-day treatment period were processed to determine the fasting plasma glucose levels.

2.3.2 Serum Insulin:

Insulin levels in the plasma were assayed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Monoclonal anti-insulin antibody, enzyme conjugate, and standard human insulin were utilized in this assay.

2.3.3 Glycosylated Hemoglobin (Hb A1C):

Hb A1C levels were estimated using a commercial kit. Hemolysate preparation, glycohemoglobin separation, and total hemoglobin estimation were conducted to calculate Hb A1C percentages.

2.3.4 Lipid Profile:

Serum lipids, including total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and VLDL cholesterol, were extracted and estimated using standard procedures. The cholesterol content in lipoprotein fractions was also determined.

These biochemical parameters provided a comprehensive overview of the metabolic and lipid profile, allowing for the evaluation of the anti-diabetic efficacy of Dichrostachys cinerea extract in the experimental rat model.

2.4 Histological Studies:

2.4.1 Tissue Collection and Processing:

Liver and pancreas tissues were carefully excised post-sacrifice by cervical decapitation. These tissues were promptly rinsed in ice-cold saline to remove extraneous elements and subsequently preserved in 10% formalin solution. Following fixation, small sections of the tissues were dehydrated using ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 6 μm thickness were then prepared for further analysis.

2.4.2 Haematoxylin and Eosin Staining:

The tissue sections underwent Haematoxylin and Eosin staining, a standard histological technique. Haematoxylin stained the nuclei blue, aiding in their visualization, while Eosin imparted a pink color to the cytoplasm and other cellular components. This staining facilitated the microscopic examination of morphological changes in liver and pancreas tissues.

2.4.3 Microscopic Examination:

Stained tissue sections were examined under a microscope to observe and document any alterations in cellular architecture, presence of inflammation, necrosis, or other morphological changes. Special attention was given to hepatocytes in the liver and islets of Langerhans in the pancreas.

2.5 Statistical Analysis:

2.5.1 Software and Hypothesis Testing:

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 7.5. Hypothesis testing methods included one-way analysis of variance (ANOVA) to compare means across different groups.

2.5.2 Post-Hoc Analysis:

Post-ANOVA, the least significant differences (LSD) test was employed for multiple comparisons between group means. This facilitated the identification of specific group variations and helped establish statistical significance.

2.5.3 Significance Level:

P-values less than 0.05 were considered indicative of statistical significance. The significance level was chosen to ensure robustness in identifying meaningful differences in the measured parameters.

2.5.4 Data Presentation:

All results were expressed as Mean ± Standard Error Mean (SEM) for the specified number of animals in each group. The statistical analysis provided a rigorous framework for assessing the significance of observed changes in the experimental parameters, reinforcing the reliability of the study findings.

3. Results:

3.1 Glucose Metabolism:

The study unveiled noteworthy enhancements in oral glucose tolerance among diabetic rats subjected to Dichrostachys cinerea treatment. These improvements were evident through a significant reduction in blood glucose levels. The results suggest that Dichrostachys cinerea extract may play a beneficial role in ameliorating impaired glucose metabolism associated with diabetes, showcasing its potential as an anti-diabetic agent.

3.2 Body Weight:

Dichrostachys cinerea extract exhibited a dose-dependent impact on the body weight variations observed in diabetic rats(5). This implies that the administered doses of Dichrostachys cinerea extract influenced the body weight of the diabetic rats in a measurable manner. The dose-dependent effect on body weight suggests a potential regulatory role of the extract in metabolic processes related to body mass. Further investigations are essential to comprehensively understand the mechanisms underlying this dose-dependent influence on body weight and to determine the optimal dosage for potential therapeutic applications.

In conclusion, the results of this study emphasize the beneficial effects of Dichrostachys cinerea on glucose metabolism and body weight regulation in diabetic rats, providing a foundation for future research exploring its potential as an anti-diabetic agent.

3.3 Serum Insulin Levels:

Dichrostachys cinerea extract demonstrated a positive influence on serum insulin levels, indicating its potential role in regulating insulin secretion(6). The observed increase in serum insulin levels suggests that the extract may have a stimulatory effect on pancreatic beta cells, enhancing insulin production. This is crucial in the context of diabetes, as insulin plays a central role in glucose homeostasis(7). The findings imply that Dichrostachys cinerea could contribute to improved insulin function, which is beneficial in managing diabetes.

3.4 Histopathological Changes:

Histological examination provided insights into the structural changes in the liver and pancreas tissues of Dichrostachys cinerea-treated rats. The results indicated a restoration of the normal architecture in both organs. In the liver, there was a reduction in degenerative changes, and hepatocytes showed signs of regaining normal size and shape. Additionally, pancreas tissues displayed improvements, with preserved islets and decreased architectural disarray. These histopathological changes suggest a protective and regenerative effect of Dichrostachys cinerea on vital organs affected by diabetes. The restoration of tissue architecture implies potential therapeutic benefits and warrants further investigation into the molecular mechanisms underlying these observed changes.

4. Discussion:

4.1 Mechanisms of Action:

The observed anti-diabetic effects of Dichrostachys cinerea extract can be linked to the presence of specific phytoconstituents identified during the phytochemical analysis. These active compounds are likely to contribute to the modulation of key mechanisms involved in insulin secretion and glucose metabolism(8).

4.1.1 Influence on Insulin Secretion:

Phytoconstituents such as flavonoids and terpenoids, identified in Dichrostachys cinerea, have been reported to exert positive effects on pancreatic beta cells, which play a crucial role in insulin production(9). Flavonoids, known for their antioxidant properties, may protect beta cells from oxidative stress, enhancing their viability and functionality(10). Terpenoids, on the other hand, have been associated with potential insulinotropic effects, stimulating insulin release from pancreatic cells(4) (11).

4.1.2 Regulation of Glucose Metabolism:

The improvement in oral glucose tolerance observed in the experimental groups suggests a role of Dichrostachys cinerea in regulating glucose metabolism. This could be attributed to the impact of phytoconstituents on glucose uptake by peripheral tissues, inhibition of gluconeogenesis, or enhancement of insulin sensitivity in target cells(12). Additionally, the observed reduction in fasting plasma glucose levels further supports the hypothesis that the extract may influence glucose homeostasis at various levels(4).

4.1.3 Antioxidant and Anti-inflammatory Properties:

Several phytoconstituents, including phytosterols and flavonoids, possess antioxidant and anti-inflammatory properties(13). Oxidative stress and inflammation are interconnected factors implicated in the pathogenesis of diabetes(14). The extract's ability to counteract oxidative stress and inflammation may contribute to the preservation of pancreatic beta cells, preventing their dysfunction(15).

Overall, the multi-faceted mechanisms of action exhibited by Dichrostachys cinerea extract, involving insulin secretion, glucose metabolism, and antioxidative properties, collectively contribute to its anti-diabetic effects. Further elucidation of these mechanisms at the molecular level through targeted studies is warranted for a comprehensive understanding of its therapeutic potential in diabetes management(16).

4.2 Comparison with Previous Studies:

Comparisons with existing literature underscore the distinctive contribution of Dichrostachys cinerea in the realm of diabetes management. While prior studies have investigated various medicinal plants for their anti-diabetic properties, the specific attributes of Dichrostachys cinerea set it apart in terms of its phytochemical composition and observed effects.

4.2.1 Phytochemical Distinctiveness:

Dichrostachys cinerea exhibits a unique phytochemical profile, as evidenced by the presence of specific compounds identified in this study. The combination of phytosterols, flavonoids, terpenoids, and other bioactive molecules distinguishes it from other plants previously explored

for anti-diabetic potential. The presence of these compounds, each with its distinct biological activities, contributes to the plant's singular pharmacological effects(16).

4.2.2 Efficacy in Glucose Metabolism:

Comparative analyses reveal that Dichrostachys cinerea demonstrates efficacy in improving glucose metabolism, as reflected in the enhanced oral glucose tolerance and reduced blood glucose levels in diabetic rats. Such outcomes surpass or, in some cases, differ from those reported for other botanical extracts. This suggests that the plant's mechanisms of action might involve unique pathways or synergistic interactions among its bioactive constituents(17).

4.2.3 Restoration of Histological Architecture:

Histopathological examination in our study indicates a restoration of liver and pancreas architecture in Dichrostachys cinerea-treated rats. This restorative effect on vital organs sets Dichrostachys cinerea apart from other herbal interventions, as not all exhibit such comprehensive impacts on tissue morphology. The observed architectural improvements align with the plant's potential to address the root causes of diabetes, extending beyond mere glucose level modulation.

In summary, Dichrostachys cinerea's distinct phytochemical composition, efficacy in glucose metabolism, and the ability to restore histological architecture contribute to its unique position in the landscape of anti-diabetic botanicals. While acknowledging the valuable insights gained from previous studies, our findings underscore the need for further research to fully unravel the therapeutic potential of Dichrostachys cinerea and its possible integration into diabetes management protocols.

5. Conclusion:

The findings of this study highlight the noteworthy anti-diabetic potential of the ethanolic leaves extract of Dichrostachys cinerea. The comprehensive investigation encompassing glucose metabolism, insulin regulation, and histological changes provides compelling evidence of the plant's therapeutic efficacy against diabetes.

5.1 Glucose Metabolism and Insulin Regulation:

The observed improvements in oral glucose tolerance and reduced blood glucose levels in Dichrostachys cinerea-treated diabetic rats underscore its significant impact on glucose metabolism. The dose-dependent effect on body weight changes further supports the plant's potential in influencing metabolic processes in diabetic conditions. These outcomes are indicative of a multi-faceted influence on glucose homeostasis, possibly through the regulation of insulin secretion or sensitivity.

5.2 Histological Changes:

Histopathological examinations revealing the restoration of liver and pancreas architecture further corroborate the anti-diabetic effects of Dichrostachys cinerea. The plant's ability to ameliorate structural changes in vital organs associated with diabetes emphasizes its potential role in addressing the underlying pathophysiological mechanisms of the disease.

5.3 Implications for Further Research:

While this study provides valuable insights, further investigations are imperative to unravel the specific compounds responsible for Dichrostachys cinerea's anti-diabetic properties. Isolation and characterization of these bioactive constituents will facilitate a deeper understanding of the plant's mechanisms of action at the molecular level.

5.4 Integration into Diabetes Management:

The significant anti-diabetic activity demonstrated by Dichrostachys cinerea positions it as a promising candidate for inclusion in diabetes management strategies. Its distinct phytochemical composition, efficacy in glucose metabolism, and ability to restore histological architecture suggest a holistic approach to addressing the complexities of diabetes.

5.5 Future Research Directions:

Future studies should focus on elucidating the exact mechanisms through which Dichrostachys cinerea modulates glucose metabolism and insulin regulation. Additionally, clinical trials are warranted to validate these findings in human subjects. Such endeavors will contribute to the development of evidence-based therapeutic interventions for diabetes.

In conclusion, the ethanolic leaves extract of Dichrostachys cinerea emerges as a compelling natural remedy for diabetes, opening avenues for further exploration and potential integration into the arsenal of anti-diabetic agents.

6. Future Directions:

The promising anti-diabetic activity exhibited by the ethanolic leaves extract of Dichrostachys cinerea paves the way for an exciting array of future research endeavors. To comprehensively understand and harness the potential of this botanical resource, the following directions are recommended:

6.1 Isolation and Characterization of Active Compounds:

Future studies should aim to isolate, purify, and characterize the active compounds present in Dichrostachys cinerea responsible for its anti-diabetic effects. Identifying these bioactive constituents will not only facilitate a more targeted therapeutic approach but also contribute to the development of standardized botanical formulations.

6.2 Mechanistic Studies:

In-depth mechanistic studies are essential to unravel the precise molecular pathways through which Dichrostachys cinerea influences glucose metabolism and insulin regulation. Elucidating the plant's mechanisms of action at the cellular and molecular levels will provide valuable insights into its therapeutic potential and guide the development of more refined interventions.

6.3 Preclinical and Clinical Investigations:

Expanding research from preclinical models to clinical trials is crucial for validating the safety and efficacy of Dichrostachys cinerea in human subjects. Well-designed clinical studies will help establish dosage regimens, evaluate long-term effects, and assess potential interactions with existing anti-diabetic medications.

6.4 Formulation Development:

The development of standardized formulations, such as extracts, capsules, or dietary supplements, will enhance the practical applicability of Dichrostachys cinerea in clinical settings. Optimizing delivery methods and dosage forms will contribute to its accessibility and ease of use.

6.5 Exploring Combinatorial Approaches:

Investigations into potential synergistic effects between Dichrostachys cinerea and conventional anti-diabetic drugs could open avenues for combinatorial therapeutic strategies. Complementary actions may enhance efficacy while potentially mitigating adverse effects associated with single-agent treatments.

6.6 Safety and Toxicology Studies:

Comprehensive safety and toxicology assessments are imperative to ensure the plant's safety profile, especially with prolonged use. Understanding any potential side effects or interactions will be pivotal for its integration into mainstream healthcare practices.

6.7 Community-Based Ethnopharmacological Studies:

Engaging in community-based studies to document traditional knowledge and practices related to Dichrostachys cinerea will enrich our understanding of its historical uses. Integrating traditional wisdom with modern scientific evidence can provide a more holistic perspective on the plant's therapeutic potential.

In conclusion, the future trajectory of research on Dichrostachys cinerea should be guided by a multidisciplinary approach, combining botanical, pharmacological, and clinical

investigations. This collaborative effort will not only advance our understanding of the plant's medicinal properties but also contribute to the development of innovative and effective strategies for diabetes management.

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