

**INVESTIGATION OF ANTIDIABETIC PROPERTIES,
MECHANISMS OF ACTION AND TOXICOLOGY OF
STRYCHNOS HENNINGSII (GILG) BARK.**

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HENNINGSII* (GILG) BARK.**

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**SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF**

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**UNIVERSITY OF FORT HARE, ALICE,
SOUTH AFRICA.**



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CO- SUPERVISOR: PROF G BRADLEY

DEDICATION

TO

GOD ALMIGHTY FOR HIS MERCY OVER MY LIFE

DECLARATION

I, the undersigned, declare that this thesis submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Biochemistry in the Faculty of Science and Agriculture, School of Biological and Environmental sciences and the work contained herein is my original work with exemption to the citations and that this work has not been submitted at any other University in partial or entirety for the award of any degree.

Name:_____

Signature:_____

Date:_____

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LIST OF ABBREVIATION

ATCC	- American Type Cell Culture
BSA	- Bovine Serum Albumin
DM	- Diabetes Mellitus
DMEM	- Dulbecco's Modified Eagles' Medium
DMSO	- Dimethyl sulfoxide
FCS	- Foetal Calf Serum
Fe	- Iron
FRAP	- Ferric Reducing Antioxidant Power
GLUT4	- Glucose Transporter 4
Hb	- Haemoglobin
LUC	- Large Unstained Cell
MCH	- Mean Corpuscular Haemoglobin
MCHC	- Mean Corpuscular Haemoglobin Concentration
MCV	- Mean Corpuscular Volume
MTT	-(3-(4,5-dimethylthiazolyl-2)- 2,5-diphenyltetrazoliumbromide)
Na ₂ CO ₃	- Sodium Carbonate
NAD	- Nicotinamide
NADH	- Nicotinamide Adenine Dehydrogenases
NADPH	- Nicotinamide adenine dinucleotide phosphatease
NaHCO ₃	- Sodium Hydrogen Carbonate
NIDDM	- Non-Insulin Dependent Diabetes Mellitus
PCV	- Pack Cell Volume

PI 3K	- Phosphatidylinositol 3'Kinase
PPAR	- Peroxisome Proliferator Activator Receptor Gamma
RBC	- Red Blood Cells
RCDW	- Red Cell Distribution Width
RPMI	- Roswell Park Memorial Institute
SH	- <i>Strychnos henningsii</i>
STZ	- Streptozotocin
TPRZ	- Tripyridyl-S-Triazine
WBC	- White Blood Cell

GENERAL ABSTRACT

GENERAL ABSTRACT

The apparent reversal of trend from modern drugs to herbal medicine is partly due to the fact that synthetic drugs have always shown adverse reactions and other undesirable side effects. Hence, the use of medicinal plants for the treatment of diseases such as diabetes is very common especially in the rural areas. Majority of these plants are used based on the experience and indigenous knowledge without identification of the therapeutic agents. There is enormous wealth of medicinal plants in the world yet many of them have not been discovered or studied scientifically to substantiate their ethno-medicinal usages.

Ethnobotanical study has been the method often used to search for locally important plant species for the discovery of crude drugs with low side effects. An ethnobotanical survey was conducted on the medicinal plants commonly used for the management of diabetes mellitus in Nkonkobe Municipality, Eastern Cape of South Africa. Information was obtained through structured questionnaire administered to traditional healers and herbalists in the region. The study revealed 15 species of plants belonging to 13 families. *Strychnos henningsii* and *Leonotis leonorus* among others were repeatedly mentioned by the traditional healers as the two mostly used plants for the management of diabetes mellitus. The infusion and decoction of the roots, leaves and barks of these plants are the methods of preparation.

The antioxidant potential of aqueous bark extract of *S. henningsii* was investigated both *in vivo* and *in vitro* using spectroscopic method. The antioxidant activity of the extract against hydrogen peroxide (H_2O_2), 2,2'-azinobis[3-

ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS), as well as reducing power was concentration dependent. The extract exhibited lower and average scavenging activities against 1,1- diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals with IC₅₀ value of 0.739 and 0.49 mg/ml respectively. The administration of the plant extract at 250, 500 and 1000 mg/kg significantly increased the activities of the antioxidant enzymes in the hepatotoxic rats induced with carbon tetrachloride. On the other hand, the stem bark extract had lower effect on lipid peroxidation level except at the dose of 250 mg/kg.

The effect of oral administration of *S. henningsii* extract was evaluated in normal Wistar rats for 28 days. The observed result indicated non- toxic effect of sub-acute administration of plant extract to the animals except at certain doses. This is because, there was no apparent damage to some haematological and biochemical parameters used in assessing organ specific toxicity. However, the alterations observed on platelet, white blood cells and its differentials imply parameter and dose selective toxicity when repeatedly consumed on daily basis at the doses investigated.

This study also investigated the antidiabetic activities of the extract at the doses of 125, 250 and 500 mg/kg body weight in diabetic rats induced with streptozotocin - nicotinamide for 15 days. The extract appreciably ($P < 0.05$) reduced the blood glucose level, feed and water intake while the best result was obtained at 250 mg/kg. Similarly, the level of triacylglycerol at the three doses investigated was significantly decreased. In addition, the glucose tolerance was reduced to near normal level after 90 min at certain doses. The clinical significance of the extract on some biochemical and haematological parameters lessen both hepatic and renal damages. Anaemic condition in diabetic animals

was also improved after plant extract administration. However, no significant effect was observed in white blood cells and some of its differentials.

The extract demonstrated strong glucose utilization in 3T3-L1 cells with a response of 278.63% of the control at 12.5µg/ml while that of Chang liver cells was 103.54%. The cytotoxicity result revealed non toxic effects of the extract to both cell lines. Treatment of 3T3 L1 cells with the extract did not reduce lipid accumulation. The extract inhibited the activity of α - glucosidase and α - amylase in a concentration dependent manner with IC₅₀ values of 38 µg/ml and 60.9 µg/ml respectively. The percentage protein antiglycation of *S. henningsii* was 18.4, 38.2 and 61.2% for 0.25, 0.5 and 1 mg/ml respectively while aminoguanidine a known inhibitor of protein glycation was 87.2% at 1 mg/ml. The FRAP assay values of the extract was 357.05 µmol Fe (II)/g. The findings from this study support the folkloric usage of this plant for the management of diabetes mellitus in the region.

Keywords: *S. henningsii*; ethnobotanical; antioxidant; toxicity; diabetes; glucose metabolism; protein glycation;

INTELLECTUAL PROPERTY AND AGREEMENT STATEMENT

All the role players including the elderly and the traditional healers who volunteered one information or the other, during the preliminary investigation on the folkloric use of *Strychnos henningsii* were adequately financially rewarded with further verbal agreement that this research shall not be for commercial purposes but will serve as an enlightenment information to the community and the entire Eastern Cape, Province on the efficacy, safety and toxicity of this plant.

ETHICAL COMMITTEE APPROVAL

The study involving the use of animals in this project was carried out following the approval of the Ethical Committee on Animal Use and Care of the University of Fort Hare.

COMPLIANCE STATEMENT

No part of this study in any form has been commercialized. The thesis is meant to be used for information dissemination on the medicinal potentials of *Strychnos henningsii* to the immediate community and the entire Eastern Cape Province of South Africa.

Supervisor signature

Student signature