BENIGN PROSTATIC HYPERPLASIA ATTENUATION AND CYTOTOXIC EFFECTS OF *Lonchocarpus griffonianus* G. DON (FABACEAE) STEM AND ROOT BARKS

BY

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A THESIS WRITTEN IN THE DEPARTMENT OF PHARMACOGNOSY AND SUBMITTED TO THE POSTGRADUATE STUDIES IN IN FULFILLMENT OF THE REQUIREMENT FOR THE DOCTOR OF PHILOSOHPY DEGREE (PhD) IN PHARMACOGNOSY OF THE UNIVERSITY OF BENIN, BENIN-CITY, NIGERIA

AUGUST, 2023

CERTIFICATION

This is to certify that this project was carried out by DANIEL AKPE-EFIAK AMBE of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin-city, Nigeria.

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Professor Buniyamin A. Ayinde Date

DVC Academic (Ekehuan campus)

(Supervisor)

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Dr (Mrs) Josephine O. Ofeimun Date

(Co-supervisor)

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Dr (Mrs) Josephine O. Ofeimun Date

Ag. Head of Department

DEDICATION

I dedicate this work to the Eternal God that granted me the grace to finish this work, and my beautiful and talented wife Pharm. (Mrs.) Ima Daniel Ambe, my gifted children (Prosperity Daniel Ambe, Akpe Daniel Ambe (Jnr) and Love Daniel Ambe).

To my late parents, Chief Akpe Daniel Ambe and Mrs. Arit Akpe Daniel Ambe

ACKNOWLEDGEMENTS

I am deeply grateful to the Almighty God for his benevolence and grace sufficient for me to finish the work. I return all the glory to him.

I am also grateful to my supervisor for sharing a part of his knowledge and experience with me. Through his tutelage, I am now a better person than I was before. Thank you, Prof.

I appreciate Dr (Mrs.) Josephine Ofeimun for her teachings, guidance, robustness and perfection during the program.

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Special appreciation goes to the Department of Pharmacognosy, University of Benin staff. My wonderful friends Mr Ugwu Kingsley, Mrs Florence Adesina, Dr Osamuyi Uwunarongie, Dr Rose Imade, Mallam Abdul-hafiz Sanni, "High Chief" Chijoke Samuel and others I have failed to mention due to space constraints.

I want to acknowledge the contributions of Dr Emmanuel Odion, the acting Head of the Department of Pharmaceutical Chemistry. He has been a wonderful mentor, a father, and a light

in the dark for me and others. May the Almighty God reward you both on Earth and in Heaven. Amen.The patience, prayers and tolerance of my wife and children are deeply acknowledged and appreciated. Those cold and long nights, loneliness and many more things you all fought without letting go due to my unavoidable absence, during the course of the program, have made me who I am today. I will not take these sacrifices for granted. May God grant you all eternal grace to reap the fruit of this seed. Amen.

Thank you Mrs. Emem Bassey Edet for your sacrifices when I was on the bench. You took the pain comes sunshine and during rainfall to get samples and lots more. May the Lord reward you bountifully. Amen.

TABLE OF CONTENT

LIST OF FIGURES **Page number**

LIST OF TABLES

ABSTRACT

Rising incidences of benign and cancerous tumours, such as benign prostatic hyperplasia (BPH) and prostate cancer, coupled with the unpleasant side effects of current therapy, suggest a need to search for new drug molecules. The stem bark of *Lonchocarpus griffonianus G. Don (Fabaceae)* is an important medicinal plant used in Nigeria to treat BPH and other tumour-related ailments. No pharmacological study on the use of the plant for treating BPH has been reported. This study aimed to investigate the protective effect of *L. griffonianus* (LG) on BPH.

Two organs (stem and root barks) of LG were identified, collected, pulverized and extracted with absolute methanol (99 %) using a Soxhlet extractor. Comparative preliminary biological evaluations were done on the *L. griffonianus* stem bark (LGSB) extract and root bark (LGRB) extracts using two benchtop assays (cytotoxic and antiproliferative). The acute toxicity of the LG stem bark extract was done using a modified Lorke's method. The extract was subjected to Vacuum Liquid Chromatography (VLC) and Gravity Column Chromatography (GCC) to obtain two isolated compounds, LO1 and LO2. The compounds were subjected to MS and 1D NMR analysis for identification. The isolated compounds (LO1 and LO2) were subjected to cytotoxic evaluation on human prostate (PC3) and uterine cervical cancer (Hela) cell lines using a 3-(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. Anti-BPH evaluation was done on the extract and LO1 using testosterone-induced BPH in the rat model. BPH was induced by the administration of testosterone propionate (4 mg/kg, s.c., in olive oil) for 28 days. LGSB extract (100, 200 and 400 mg/kg), LO1 (5 mg/kg), LGSB extract (200 mg/kg)+finasteride (5 mg/kg) and finasteride (5 mg/kg) were orally administered daily. On day 29, the rats were sacrificed under anaesthesia and blood was collected via the abdominal aorta. The collected blood was centrifuged, and the serum was separated. The serum was analyzed for biochemical parameters such as prostate-specific antigen (PSA), testosterone and estradiol. The prostate was harvested for histological examination. The wet weight and volume of the prostate were taken. The prostate index (PI) was calculated. All data were expressed as mean ± SEM (standard error of the mean) and were compared using analysis of variance (ANOVA),

The result of preliminary evaluations indicated that the LGSB extract has a higher activity (100 ± 0.00% mortality at 80 µg/mL) than the LGRB extract (3.33 ± 1.29% at 80 µg/mL). Acute toxicity results revealed no mortality in both phases after oral administration with LD50>5000 mg/kg. LO1 and LO2 significantly inhibited the multiplication of PC3 and Hela cells *in vitro*.

The LGSB extract treatment significantly ($p<0.0001$) reduced prostate weight, volume and prostate index in rats induced with testosterone. There was a significant ($p<0.001$ and $p<0.0001$) lowering of testosterone in the serum of the animals. The dose of 100 and 400 mg/kg body weight of the extract caused a 79.26% (0.28 ± 0.06) reduction in the testosterone concentration of rats serum. The Prostate-Specific Antigen (PSA) level in the serum was significantly ($p<0.01$ and $p<.001$) reduced in comparison with the negative control. The dose of 400 mg/kg body weight of the extract caused a 70.28% (0.74 ± 0.18) reduction in the PSA of rats. There was no significant difference in the estradiol result obtained for the study. The histopathological presentation revealed that the extract ameliorated testosterone's effect on prostate histomorphology's architecture. The results of the study demonstrated that the crude extract and lupeol isolated from the extract attenuated rat prostate enlargement. Two bioactive phytosterols (LO1 and LO2) were characterized as lupeol and β-sitosterol and showed cytotoxic activity against human prostate and uterine cervical cancer cell lines. The ethnomedicinal usage of *L. griffonianus* for the treatment of BPH was validated by this study.