ISOLATION, CHARACTERIZATION AND IMMUNOCHEMICAL STUDIES OF PROTEINS FROM THE SHELL OF *CYPRAEA MONETA* (*LINNAEUS, 1758*) (COWRY SHELL)

BALOGUN, RODIAT OLUSOLA

2012

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BY

BALOGUN, RODIAT OLUSOLA

B.Sc (MICROBIOLOGY), IFE.

BMSP08/09/2913.

A THESIS SUBMITTED TO THE DEPARTMENT OF HAEMATOLOGY AND IMMUNOLOGY, FACULTY OF BASIC MEDICAL SCIENCE, COLLEGE OF HEALTH SCIENCES, OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc) DEGREE IN IMMUNOLOGY.

2012

CERTIFICATION

We certify that this work was carried out by BALOGUN, Rodiat Olusola, BMSP08/09/2913**,** in the Department of Haematology and Immunology, Faculty of Basic Medical Sciences, College of Health Science, Obafemi Awolowo University, Ile-Ife.

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Dr. (Mrs.) R. A. Togun — (Supervisor)

Dr. (Mrs) N. O. Akinola — (Head, Department of Haematology and Immunology)

DEDICATION

This work is dedicated to Almighty Allah, The Gracious, The Merciful and Prophet Muhammad (S.A.W), the mercy to mankind.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisor, Dr. (Mrs) R. A. Togun, for her guidance, encouragement, patience, understanding, thoroughness and immeasurable input to the success of this work. She is really a great mother.

I am also grateful to Dr. R. E. Okonji for providing me the research facilities for this study and imparting valuable knowledge into my life. My appreciation also goes to Prof. M. A. Durosimi, Dr. L. Salawu and Dr. (Mrs) N. O. Akinola for their concern and contributions towards this work. My sincere gratitude also goes to Mr L. O. Akinyemi, for his profound supports on this research, Mrs A. O. Adeloye, Mrs A. F. Obadire, and Mr S. O. Fasae for their great support in the cause of this work.

Special thanks are also extended to Dr. E. M. Obuotor, Dr. (Mrs) A. Kuku, Mr O.O. Odekanyin, from the Department of Biochemistry, Mr D. O. Adeyemi from the Department of Anatomy for their valuable assistance and constructive advice and Dr T. A. Esan from Dentistry. My heartfelt thanks go to Dr (Mrs) L. M. Durosinmi (D.S.A), Dr O. K. Owolarafe and Dr. L. Umar for their wonderful support to the success of this work.

Special appreciation goes to my colleagues; Mrs Salaudeen Olayinka, Bolanle Ayanda, Damilola Ayanbadejo, Bukola Olofinjana, Yetunde Bulu, and my roommate Adaeze Onumejor. My sincere gratitude and appreciation goes to my dear friends, Mrs S. Okikiola Azeez, for her assistance, encouragement and support throughout this work.

I am greatly indebted to my Parents, Alhaji S.O. Balogun, Mummies; Kekere and Agba, for their effort and contributions to my education and well-being. One cannot ask for better parents than you. My thanks also goes to my siblings: Adunola, Dasola, Ibidolapo, Opeyemi, Ikeoluwa, Modupe, Olufunke, Olabisi, Faridah, Tunde and Hamid, my mother in-law for their love, care and prayer.

Special thanks go to my friend, brother, Onifade Shakiru Abayomi Idowu (OSAINET), love of my life for his encouragement and support throughout this research work.

My utmost gratitude goes to the Almighty Allah (SWT) and HIS Prophet Muhammed (PBUH). HE is my Help from past and my Hope for years to come. In Allah I put my trust.

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ABBREVIATIONS

CSP – Cowrie Shell Protein

PCV – Packed Cell Volume

WBC – White Blood Cell count

Bw – Body weight

H &E - **Hematoxylin and Eosin stain**

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Abstract

Cowry shells have recently become the focus of several research efforts because of their high content of chitin and chitosan, which have many important biomedical potentials. However many of these applications are potential sources of immune activation. Hence, there is a need to characterize the protein content and investigate the immunological properties of cowry shell.

Proximate analysis of the powdered shell was carried out according to the procedure of the Association of Official Analytical Chemistry (A.O.A.C 2006). Protein was extracted with Phosphate Buffered Saline (PBS) or Citrate buffer according to standard procedure. Protein concentration was determined using Bradford method. The crude protein extract was separated and purified by gel filtration techniques. Degree of purity was ascertained by using non-denaturing polyacrylamide gel electrophoresis (PAGE). The native and subunit molecular weights were determined by gel filtration and SDS-polyacrylamide gel electrophoresis, respectively. Immunological studies were performed with serum containing cowry shell antibody obtained from rabbits using Ouchterlony and Immunoelectrophoresis techniques. Group B, C, and D of mice was injected intraperitoneally with cowry shell protein of 10, 100, and 1000mg/kg body weight doses, respectively and the control with citrate buffer (group A). Changes in body weight, packed cell volume (PCV) and white blood cell (WBC) counts were monitored daily for two weeks, after which histopathological studies were performed on the organs liver, kidney, spleen, and lungs.

The results showed the proximate analysis of powdered cowry shells as follows: Moisture content (0.74%); Nitrogen content (0.30%); Ash content (88.82%); Crude fibre (4.38%); Crude protein (1.91%) and Crude fat (0.18%). Gel filtration of the crude extract of powdered cowry shell on Sephadex G-150

showed two widely separated peaks that were also resolved in the process. The native molecular weights of the two peaks, determined by gel filtration, were 87,000 Da and 31,000 Da, respectively. Non-SDS-polyacrylamide gel electrophoresis (PAGE) of the crude extract showed two bands. Each of the two peaks pooled from gel filtration showed single bands on SDS- polyacrylamide gel electrophoresis (PAGE) with 19,000 Da and 19500 Da respectively. The serum containing cowry shell antibodies obtained from rabbits immunized with the crude extract precipitated the antigen in double immunodiffusion test. Immunoelectrophoresis of the crude extract showed precipitation of the protein only at the point of application, without any separation into bands.

There were no significant differences between control mice and those injected with different concentrations of proteins, for PCV, WBC counts but there was significant difference in body weight of group B mice, compared to control. By physical observation no morphological or behavioral changes and no death of mice were recorded throughout the experimental period. Histopathological studies revealed visible damaging effects of the cowry shell protein on the liver, kidney, lung and spleen.

It could be concluded that cowry shells contain low protein contents which did not manifest toxicity at low doses, but causes visible organ damage to some organs at high concentrations, suggestive of possible organ damage without concurrent physical manifestations in the mice.

CHAPTER ONE

1.0 Introduction

1.1 Cowrie

Cowry shells also known as "Cowries" are the hard outer protective layer of marine organisms found along shorelines and beaches when they are washed ashore by the tide (The Nation, 2011). Cowries generally belong to the phylum *Mollusca,* and family *Cypraeidae* (Linnaeus, 1758). The superfamily Cypraeacea, includes three main families: Ovulidae (egg shells), Triviidae (includes the British "cowries") and Cypraeidae (the "true" cowries) (Julian, 2005). The marine gastropod family Cypraeidae is composed of some 220 recent species and more than 500 extinct species (Kay, 1996) with species occurring worldwide. They are chiefly found in tropical regions especially around the Maldives, East Indian and Islands in the Indian Ocean (Poutiers, 1998).

The secretion of a shell by molluscs is a striking example of a self assembling process performed outside living tissues. When a mollusc builds its shell, the calcifying epithelium of its mantle extrudes mineral ions, mainly calcium and bicarbonate. In addition, it secretes an extracellular matrix composed of proteins, glycoproteins, proteoglycans, and polysaccharides (Simkiss *et al*., 1989). The shell of a cowry is typically domed, with a flat base and almost bilaterally symmetrical. It is usually solid and glossy, and often very colourful. They range in size, and the small shell is approximately 30 to 45 mm long (Poutiers, 1998), depending on the species.

The money cowrie, *Cypraea moneta*, is both abundant in nature and an excellent herbivore. A similar species, the ring cowrie, *Cypraea annulus* (Figure 1.1), is likewise

exceptionally common in many shallow reef environments, and is also herbivorous (Houbrick, 1978). These two species are small, about an inch in length, and do very well in marine aquaria. Tiger Cowries, (*Cypraea tigris*), are also good herbivores.

Cowries are easily distinguished from shells of other snails. Their shell is basically ovoid, with the aperture on the bottom. This aperture is a slender opening, and lined on both sides by calcareous bumps or nodules. The shells are highly polished and often brightly coloured, but the mantle that comes out and covers the shell when the animal is moving is often even more brightly coloured (Fretter, 1994).

Cowries are favourites of collectors because of their beautiful colours. The mantle is usually ornamented with papillae that provide camouflage and assist in respiration. The colour of the mantle sometimes matched the sponges it feeds upon. (Harasewych, 1991). The fascinating colour observed in cowry shells can be attributed to various chemical and structural features. Different colours of cowries are dependent on the presence or absence of aluminium compounds and the acidity of the soil (Helman, 2002). The abnormalities usually observed in cowry shells are overproduction of shell in the mantle.

1.2 Importance of Cowry shell

The most important function of an external shell is to protect the animal from the elements of its habitat and attacks by predators. Deadly venoms of some cowrie shells used to help victims of strokes and heart diseases and to produce a revolutionary new drug for chronic pain control (Helman, 2002).

Figure 1.1 Left: Ring cowrie, *Cypraea annulus*. Right: *Cypraea moneta* (Houbrick, 1978).

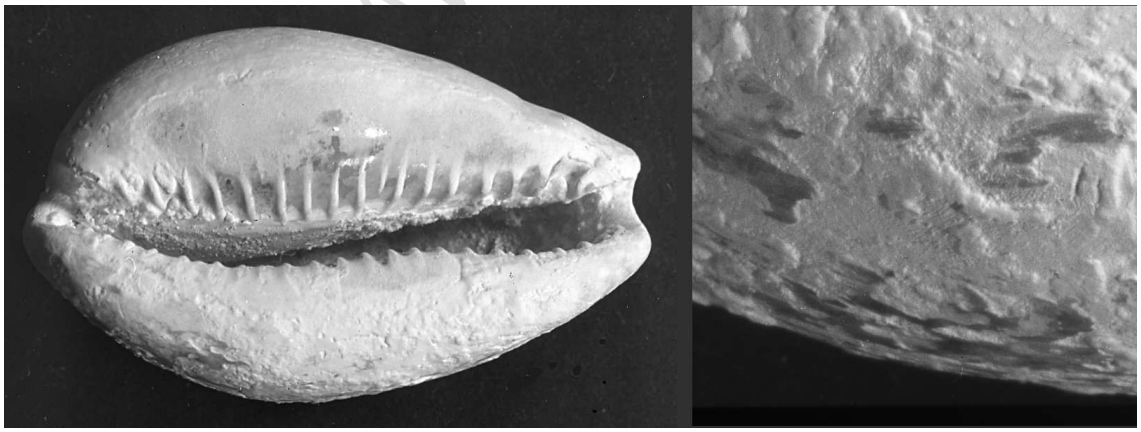

Figure 1.2: A cowrie shell. The close up on the right shows fragments of the original surface 1mm above the level of the corroded parts. This is typical of marine shells stored in wooden cabinets. The corrosion layer is calcium acetate and calcium formate. (Padfield *et. al*., 1982)