BIOCHEMICAL EFFECTS OF ROOT-BARK EXTRACT OF *Theobromacacao* (Linn.) ON LIPID PROFILES AND OXIDATIVE ENZYMES OF RATS FED WITH HIGH SALT-DIET

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CERTIFICATION

This study was approved in accordance with the partial fulfillment of the requirements for the award of Master of Science (M.Sc.) Degree in Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

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Prof. O.O.Oyedapo Date

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Head of Department.

DEDICATION

This piece of work is dedicated to the Blessed Memories of My Later Mother (Mrs. Julliet Ugochi Onwumelu) and My Late Sister (Angela Uloma Onwumelu) for the encouragementand the foundation that they laid for me before joining their ancestors.Mama and Daa, may your souls rest in the Bosom of Our Lord Jesus Christ until we meet to part no more. Amen.

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ABSTRACT

This study evaluated the *in vitro* antioxidant and anti-inflammatory potentials of aqueous root-bark extract of *Theobroma cacao* and its fractions in rats fed with high-saltdiet. This was with a view to explore its usefulness in the management of high-saltrelated ailments.

Fresh root-bark of *T. cacao* (750 g) was extracted with 6.0 L of hot distilled water for 24 h and followed by evaporation under reduced pressure at 40°C to obtain a residue (brown- flake) named aqueous extract (AqE). This was phytochemically screened and partitioned with solvents of increasing polarity to give hexane fraction (n-HF), ethyl acetate fraction (EAF), butanol fraction (n-BF) and aqueous fraction (AqF). The total phenolic and flavonoid contents of AqE and fractions were quantified. Thirty (30) albino rats were grouped into six (6) groups of 5 rats each. Group I served as control; groups II and III received salt-free diet and water with 250 and 500 mg/kg body weight(bwt) EAF. Group IV and V received 4 % (w/w) salt diet and 1 % (w/v) salt waterwith 250 and 500 mg/kg bwt EAF, while group VI received only 4% (w/w)salt diet and 1% (w/v)salt water constantly for 21 days.Biochemical changes as a result of exposure to salt-diet, salt water and EAF were investigated for 21 days. The effects of EAF on liver marker enzymes, lipid profiles as well as on the activities of oxidative enzymes were evaluated in the plasma and liver homogenates of rats. The histopathological evaluation of the kidney tissues was also carried out.

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, triterpenes, steroids/phytosterols, saponins and xanthoproteins. The phenolic content of AqE was 133.79 ± 0.02mgTAE/g (tannic acid equivalent) and its fractions ranged between 0.82 ± 0.07 and 50.93 ± 0.13mgTAE/g, while the flavonoid contents of AqE was 304.13 ± 0.13 mgRE/g (rutin equivalent) and the fractions ranged between 8.36 ± 0.11and 458.25 ± 0.16 mg RE/g. The fractions exhibited potent ferric reducing antioxidant power (FRAP). The fractions exhibited potent and appreciable DPPH radical scavenging activities and compared favourably with the standard(ascorbic acid). The fractions exhibited appreciable anti-inflammatory properties and compared favourably with the standard drug (Acetaminophen). There were significant differences ($p < 0.05$) in liver marker enzymes (ALT and AST) activities, total protein, total cholesterol, triacylglycerol, HDL-c, LDL-c and VLDL-c concentrations in the plasma and liver of treated animals. In addition, there were significant differences ($p < 0.05$) in

the liver GSH, GPx andlipid peroxidation activities of the treated animals, although there were no significant differencesin the liver SOD and CAT activities of the treated animals, when compared with the control group. Histologically, 250 mg/kg bwt EAF protected the kidney tissues of the animals from renal dysfunction, caused by consumption of high-salt diet.

The study concluded that the root-bark extract of *T.cacao* contained a broad spectrum of bioactive compounds that exhibited potent, significant and appreciable anti-inflammatory and antioxidant activities. It also reversed metabolic derangement associated with consumption of high salt-diet and water by experimental animals.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Medicinal Plants

Plants have evolved the ability to synthesize bioactive compound that are capable of defense against predators such as Cyanidine-3-*O*-glucoside and isoflavonoids (against insects and fungi) (Hedin and Waage, 1986; Lee and Gould, 2002), isoflavonoids (against herbivorous mammals), Anthocyanins (for production of flower color and attraction of animal vectors for pollination and seed dispersal, hydroxycinamic acids(for protection of plants from UV-rays). They form the basis for allelopathic interactions with other plant species and by chance, some of these compounds while being toxic to plant predators, turn-out to have beneficial effects when used to treathuman diseases (Fabricant and Farnsworth, 2001). These plant chemicals (phytochemicals) are known as secondary metabolites. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. The Primary constituents include the common sugars, amino acids, proteins,nucleic acids, chlorophylls etc.The secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolic (derived from carbohydrates). The major secondary metabolites include saponins, tannins, steroids, flavonoids, cardiac glycosides and phlobatanins (Osagie and Eka, 1998).Many of the herbs and spices used by humans (as seasonings)yield useful medicinal compounds (Tapsell *et al.*, 2006). Similar to prescribed drugs, a number of herbs are thought to cause adverse effects, however, adulteration, inappropriate formulation, or

lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal (Elvin-Lewis 2001; Lai and Roy, 2004).

1.2: Pharmaceutical Substances of Plant Origin

The vast bulk of early medicinal substances were derived from plants. An estimated 3 billion peopleworldwide use traditional plant medicines as their primary form of healthcare (Wash, 2000; Oyvind and Kenneth, 2006). At least 25% of all prescribed drugs sold in Nigeria contain active substances, which were originally isolated from plants (or are modified forms of chemicals, originally isolated from plants) (Drew 1993, Olson and Ratzkin, 1999). Plants produce a wide array of bioactive molecules via secondary metabolic pathways. While some of these compounds are directly extracted from plant materials; chemical modifications of many of these plant-derived drugs have yielded a range of additional therapeutic substances. The bulk of plant-derived medicines arecategorized into a number of chemical families, which include, alkaloids, flavonoids, terpenes and terpenoids, steroids (e.g., cardiac glycosides), as well as coumarins, quinines, salicylates and xanthines (Walsh 2000) (Table 1.1).

Table 1.1: List of Some Plant-Derived Drugs

Drug	Chemical type	Indication	Plant Source/origin
Aspirin	Salicylate	Analgesic,anti-inflammatory	*Salix alba* (white willow tree and *Filipendula ulmaria* (meadowsweet)
Atropine	Alkaloid	Pupil dilator	*Atropa belladonna* (deadly nightshade)
Caffeine	Xanthine	Increases mental alertness	*Camellia sinensis*
Cocaine	Alkaloid	Ophthalmic anaesthetic	*Erythoxylum coca* (coca leaves)
Codeine	Alkaloid	Analgesic, cough suppressor	*Papaver somniferum* (opium poppy)
Dicoumarol	Coumarin	Anti-coagulant	*Melilotus officinalis*
Digitoxin	Steroid	Increases heart muscle contraction	*Digitalis purpurea*
Ipecac	Alkaloid	Induces vomiting	*Psychotria ipecacaunha*
Morphine	Alkaloid	Analgesic	*Papaver somniferum* (opium poppy)
Pseudoephedrine	Alkaloid	Clears nasal congestion	*Ephedra sinica*
Quinine	Alkaloid	Malaria	*Cinchona pubescens* (fever tree)
Reserpine	Alkaloid	Antihypertensive (reduces blood pressure)	*Rauwolfia serpentine* (Indian snakeroot)
Scopalamine	Alkaloid	Motion sickness	*Datura stramonum* (Jimson weed)
Taxol	Terpenoid	Ovarian, breast cancer	*Taxus brevifolia* (wester yew tree)
Theophylline	Xanthine	Anti-asthmatic, diuretic	*Camellia sinensis*
Vinblastine/vincristine	Alkaloid	Hodgkin's disease/Leukaemia	*Catharanthus roseus* (rosy periwinkle)

Adapted fromWalsh (2000).

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