HYPOCHOLESTEROLEMIC AND THERAPEUTIC EFFECT OF *Moringa oleifera* LEAVES AND ITS EXTRACT ON RATS

By

MOHAMMED ABD EL-HAMID AWAD GHALY
B.Sc. Agric. Sci. (Food Science), Fac. Agric., Al-Azhar Univ., 2011

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF FOOD SCIENCE

In

Agriculture Science
(Food Science)

Department of Food Science
Faculty of Agriculture
Cairo University
EGYPT

2017

APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. AKILA SALEH HAMZA.....
Emeritus Head Researcher, Regional Center for Food and Feed, Agriculture Research Center

Dr. FERIAL SAYED AHMED EL-HASHIMY.....
Emeritus Professor of Food Science, Fac. Agric., Cairo University

Dr. NASHWA FATHY SAYED MORSY.....
Associate Professor of Food Science, Fac. Agric., Cairo University

Dr. SHAHINAZ AHMED HELMY MOHAMED.....
Professor of Food Science, Fac. Agric., Cairo University

Date: / /

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. SHAHINAZ AHMED HELMY MOHAMED
Professor of Food Science, Fac. Agric., Cairo University

Dr. NASHWA FATHY SAYED MORSY
Associate Professor of Food Science, Fac. Agric., Cairo University

Dr. SHAHENDA MOHAMED MAHMOUD ELABY
Senior Researcher, Regional Center for Food and Feed, Agriculture Research Center

Name of Candidate: Mohammed Abd El-Hamid Awad Ghaly **Degree:** M.Sc.
Title of Thesis: Hypocholesterolemic and Therapeutic Effect of *Moringa oleifera* Leaves and its Extract on Rats.
Supervisors: Dr. Shahinaz Ahmed Helmy Mohamed
Dr. Nashwa Fathy Sayed Morsy
Dr. Shahenda Mohamed Mahmoud Elaby
Department: Food Science **Branch:** Food Science **Approval:** / /

ABSTRACT

The major symptom for heart diseases and atherosclerosis is hypercholesterolemia. Based on assumption, *Moringa oleifera* leaves and its extract are used for prophylaxis (protection) against cardiovascular system diseases. The present investigation has been conducted to study the protective effect and anti-atherosclerotic effect of moringa leaves and its extract on experimental rats fed on high fat diet. In addition to evaluate the therapeutic effect of a diet containing moringa leaves or its extract on serum lipids content and liver enzyme activities as well as measure the blood lipid peroxidation of high cholesterolemic rats (HCR). Gross chemical composition of moringa leaves was determined. Polyphenols content and fractionation were obtained. Concerning, the biological evaluation, sixty adult male albino rats weighing 188±15.21 g were divided into six groups. The rats were fed on high fat diet (HFD) containing (17% lard and 3% corn oil +1% cholesterol + 0.25% bile salts) for two months, plus two concentrations of moringa leaves powder, 0.737% (containing 29.13 mg gallic acid equivalent (GAE)/100 g diet) and 1.475% moringa leaves powder (containing 58.13 mg (GAE)/100 g diet), or orally administered with 200 mg moringa leaves ethanolic extract (containing 15.5 mg GAE/kg body weight of rat) and 400 mg moringa leaves ethanolic extract (31 mg GAE/kg body weight of rat). Biochemical analysis of blood serum including total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, total lipids, liver function enzymes and atherogenic index as well as liver weight percent of rats were determined.

Results indicated that feeding rats on HFD led to increase serum lipid levels, liver weight (%) and also increase liver function enzymes (harmful effect) in serum. Results also ascertained that consumption of moringa leaves or its extract can reduce the bad effect of feeding (HFD) by lowering lipid profile levels and liver function enzymes.

Also, to investigate the therapeutic potency of moringa leaves or extract, two concentrations were chosen, 0.737% moringa leaves powder and 400 mg moringa leaves ethanolic extract/kg body weight, were given to hypercholesterolemic rats (>240 mg/dL) and basal diet, compared with a group of rats given hypercholesterolemic rats for the rest period and another group of rats given fluvastatin as hypocholesterolemic drug (2 mg/kg body weight of rat), for 27 days. Our findings proved that feeding either moringa leaves powder or its extract as well as fluvastatin significantly decreased serum lipids, liver function enzymes, lipid peroxidation rate and liver weight (%), compared with HCR ($p \leq 0.05$). However, fluvastatin increased liver function enzymes in serum of rats (as its side effect). Based on the results of our investigation, feeding on moringa leaves powder at 0.737% or administering its ethanolic extract (400 mg) have protective and therapeutic effective role in heart and cardiovascular diseases.

Key words: *Moringa oleifera* leaves, ethanolic extract, hypercholesterolemic, serum lipids, liver function enzymes.

DEDICATION

I dedicate this work to my parents and brothers for all their support, patience, kindness and encouragement during my post-graduate studies.

ACKNOWLEDGEMENT

I wish to express my deepest gratitude and sincere thanks to ***DR. Shahinaz A. H. Mohamed,*** *Professor of Food Science, Faculty of Agriculture, Cairo University, for her proper selection of the subject, valuable suggestions, kind supervision, guidance, valuable advice, infinite support through this work and for her revision of the manuscript of this thesis.*

I am very grateful to ***DR. Nashwa F. S. Morsy****, Associate Professor of Food Science, Faculty of Agriculture, Cairo University, for suggesting the points, providing the facilities of lab experimental work, infinite supports sincere guidance supervision on this study, generous help and indispensable comments.*

Any word fails to express my gratitude and deep indebtedness to ***DR. Shahenda M. M. Elaby****, Senior Researcher, Agriculture Research Center, Regional Center for Food and Feed, for her kind help, fruitful advice, encouragement, construction and generous support that providing of all facilities for producing this work, without this meteoritic support, this work will abort.*

LIST OF ABBREVIATIONS

Alanine aminotransferase	**(ALT)**
Analysis of variance	**(ANOVA)**
Aspartate aminotransferase	**(AST)**
Association of official analytical chemists	**(AOAC)**
Atherogenic index	**(AI)**
Cholesterol esterase	**(CHE)**
Cholesterol oxidase	**(CHO)**
Gallic acid equivalent	**(GAE)**
Glycerol kinase	**(GK)**
Glycerol-3-phosphate-oxidase	**(GPO)**
High density lipoprotein cholesterol	**(HDL-C)**
High fat diet	**(HFD)**
High performance liquid chromatography	**(HPLC)**
Hypercholesterolemic rats	**(HCR)**
Institutional animal care and use committee	**(IACUC)**
International unit	**(IU)**
Lactate dehydrogenase	**(LDH)**
Lipoprotein lipase	**(LPL)**
Low density lipoprotein cholesterol	**(LDL-C)**
Malate dehydrogenase	**(MDH)**
Malondialdehyde	**(MDA)**
Methanolic extract of *Moringa oleifera* leaves	**(MEMOL)**
Nicotinamide adenine dinucleotide-hydrogen	**(NADH)**
Peroxidase	**(POD)**
Revolution per minute	**(rpm)**
Standard deviation	**(SD)**
Thiobarbituric acid	**(TBA)**
Total cholesterol	**(TC)**
Total lipids	**(TL)**
Total phenolic content	**(TPC)**
Triglycerides	**(TG)**
Very low density lipoprotein cholesterol	**(VLDL-C)**
World health organization	**(WHO)**

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INTRODUCTION

Nowadays, the demand of the functional foods, nutraceutical, pharmaceutical and cosmetic industries of new sources of bioactive compounds is increasing together with the public interest in herbal medicines and natural products (Rodríguez-Pérez *et al.,* 2016). *Moringa oleifera* is a member of the Moringaceae family, known as drumstick tree or miracle tree, native to India and Pakistan, which grows naturally at moderate altitude. Currently, moringa plants are widely cultivated in the Middle East, Africa and Southern Asia as a multipurpose crop (Sánchez *et al.,* 2006 ; Nouman *et al.,* 2014 and Sadek, 2014). The ancient Romans, Greeks, and Egyptians utilized this plant. Almost all parts of the plant (seeds, leaves, oil, sap, bark, roots, and flowers) are used culturally for its nutritional value and supposed medicinal properties and for flavoring as a vegetable and seed (Kasolo *et al.,* 2010 and Stohs and Hartman 2015). In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutritional content (Thurber and Fahey, 2009; Mbikay, 2012 and AbdullRazis *et al.,* 2014).

The leaves of *M. oleifera* can be eaten fresh or cooked, and reports have shown that it can be stored as a dried powder for many months without any major loss of its nutritional value (Arabshahi *et al.,* 2007). Earlier studies have found *M. oleifera* to be non-toxic and recommended for use in developing countries (Mangale Sapana *et al.,* 2012). *M. oleifera* leaves was approved as a new food resource by the ministry of health of the people's republic of China for its high nutritional value (Qi *et al.,* 2016). Extraction of plant materials depends

on various factors such as solvents, methods, and extraction time to separate the different quality and quantity of bioactive components in the crude extracts (Hayat *et al.,* 2009). In addition, the nature of the sample matrix and the compounds to be extracted also substantially affect the efficiency of extraction (Mustafa and Turner, 2011). However, to date the biological activity and medicinal functions of moringa extracts has been mainly supported by *in vitro* assays based upon their antioxidant capacity and bioactive profile (Anwar *et al.,* 2007 and Sultana *et al.,* 2009). The leaves contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani *et al.,* 2013 and Vongsak *et al.,* 2014). Various preparations of *M. oleifera* are used for their anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, anti-diabetic (Anwar *et al.,* 2007; Mbikay, 2012 and AbdullRazis *et al.,* 2014). Data available on the phytochemical composition of moringa plants suggest that the polyphenolic content of moringa is closely dependent on the germplasm considered, maturity stage, and agro climatic conditions (Anwar *et al.,* 2006 and Nouman *et al.,* 2014).

Atherosclerosis is a disorder of blood vessels, which preferentially affects the large and medium-sized arteries and therefore, generally called as "hardening of the arteries". The main arteries affected are the aorta, coronary artery and it may also affect capillaries after a prolonged period (Mallika *et al.,* 2007). Atherosclerosis referred to as a silent killer, is one of the leading causes of death in the developed countries and is on the rise in developing countries like India (Ghatak and Asthana, 1995). The American heart association has

identified the primary factors associated with atherosclerosis as elevated levels of cholesterol and triglycerides in the blood (Rajanandh *et al.,* 2012). World health organization (WHO) predicted that heart diseases and stroke are becoming more deadly, with a projected combined death toll of 24 million by 2030. Moreover, the current predictions estimate that by the year 2020, cardiovascular diseases, notably atherosclerosis will become the leading global cause of total disease burden (Chumark *et al*., 2008). Hyperlipidemia is the term used to denote raised serum levels of one or more of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or both total cholesterol and triglyceride (combined hyperlipidemia) (Sivaiah and Reddy, 2012). High dietary fat intake promotes the development of obesity in humans and rodents because of an imbalance between energy intake and energy expenditure (Bray *et al.,* 2004). Many anti-hyperlipidemic agents like a statin, fibrates, niacin, bile acids, and ezitimibe, *etc*., reduce cholesterol level with different conditions (Durrington, 2003). There is a therapeutic dilemma about the use of statins in the treatment of dyslipidemia and associated conditions, particularly in the presence of elevated liver aminotransferase levels (Calderon *et al.,* 2010). The administration of the crude leaves extract of *M. oleifera* decreases cholesterol levels in serum, liver, and kidney in the rats fed a high fat diet (HFD) (Ghasi *et al*., 2000). The hypolipidaemic effect of the ethanol leaves extract of *M. oleifera* mainly in terms of reduction in serum lipids and body weight loss of rats (Atsukwei *et al.,* 2014). So, in some Northern country, encapsulated moringa powder is already commercialized by