

Therapeutic Effect of *Moringa oleifera* Leaves and Its Extract on Hypercholesterolemic Rats

Shahinaz A. Helmy¹, Nashwa F. S. Morsy^{1*}, Shahenda M. Elaby²,
Mohammed A. A. Ghaly²

¹Food Science Department, Faculty of Agriculture, Cairo University, Egypt

²Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt

Abstract: This study was carried out to evaluate the therapeutic effect of moringa leaf powder and its ethanolic extract as a hypolipidemic agent in hypercholesterolemic rats (HCR) fed on a basal diet for 27 days. Results showed that moringa leaf powder and its extract had a significant effect in the reduction of total cholesterol, low density lipoprotein, total lipids, atherogenic index, triglycerides, alanine aminotransferase, aspartate aminotransferase and malondialdehyde of hyperlipidemic rats in comparison with the standard drug (fluvastatin). Moringa leaf powder and its ethanolic extract enhanced the increase in high density lipoprotein. Moringa leaf powder could be used as a natural source of polyphenols. Since, moringa leaf powder was superior than its extract as hypolipidemic agent.

Keywords: Moringa leaf powder, moringa ethanolic extract, hypercholesterolemic, fluvastatin, rats.

I. Introduction

Hypercholesterolemia or excessively high plasma cholesterol levels, is a strong predictor and contributory risk factor in the development of atherosclerosis-related diseases such as ischemic heart diseases, carotid artery diseases, and hypertension. According to WHO, by 2030 there will be 23.6 million people globally affected by cardiovascular diseases (CVD), making it the leading cause of mortality worldwide [1]. Currently, drugs like statins are used for lowering cholesterol, however, numerous side effects arise with its use, including muscle damage, liver dysfunction, and increased blood glucose levels [2].

Herbal medicine is still the most abundant, affordable, reliable, trusted and well understood form of health care in virtually all African villages [3] and 80% of African populations use some form of traditional herbal medicine [4,5]. *Moringa oleifera* is the widely cultivated species of the Moringaceae family in several Asian and African countries [6]. It is commonly known as drumstick tree [7]. Recent years, *M. oleifera* has attracted great attention among researchers because of the potential use in many fields. Almost every part of the tree can be eaten, and more important thing is that all parts of the tree have great potential as medicines [8]. The leaves can be integrated in salad, traditional sauces, or soups in raw or processed form; young leaves can also be steam cooked [9]. Therefore, many research workers paid great attention to the medicinal and nutritional uses of *M. oleifera* [10]. Various parts of the plant act as cardiac and circulatory stimulants, possess antitumor [11], antipyretic, antiepileptic, anti-inflammatory, and antiulcer properties [12]. The consumption of the leaves could fulfill the recommended daily requirements of several macro and micronutrients. In fresh or dried forms, the addition of drumstick leaves increases the content in iron, proteins and vitamin A of traditional dishes [13]. In animal models (gerbil, mouse), the carotene bioavailability was high with raw and dehydrated drumstick leaf consumption [13,14]. *M. oleifera* leaf contains lots of nutrients which can be absorbed into human body, such as vitamins, minerals, and fatty acids [15]. Additionally, the leaf has been certified to contain various compounds like flavonoids, phenolics, and carotenoids which can be used as antioxidant [16,17].

The present study aimed to evaluate the therapeutic effect of *M. oleifera* leaf powder and its extract on hypercholesterolemic rats.

II. Materials And Methods

2.1 Plant material

Fresh leaves of *M. oleifera* were collected during the 2013 season. The leaves were identified and authenticated at the Egyptian Scientific Society of Moringa, Cairo, Egypt. Fresh leaves of *M. oleifera* were dried and grounded and the powder obtained was stored in a dry place in the dark.

2.2 Chemicals and Reagents

Folin-Ciocalteu phenol reagent, gallic acid, and bile salts were purchased from Sigma-Aldrich (St. Louis, USA). Cholesterol, casein and cellulose were purchased from PanReacAppliChem GmbH (Germany), Loba Chemie Pvt Ltd (India) and El-Gomhoria Pharmaceutical Company (Cairo, Egypt), respectively. Sugar, corn oil, starch and lard were purchased from the local market, Egypt.

2.3 Extraction of polyphenols from moringa leaves

Extraction of polyphenols from *M.oleifera* leaves was performed at room temperature according to [18] by aqueous ethanol (70%), using an extraction ratio of 1:20. The solution was stirred at 300 rpm for 30 min. After filtration, the extract was kept in the dark at -20°C . The ethanolic extract was concentrated at 40°C in a rotary evaporator and the semisolid material obtained was stored at -20°C to avoid compound degradation for the biological experiment.

2.4 Determination of total phenolic content of moringa leaf powder and its extract

Total polyphenols was determined according to [19] using the Folin-Ciocalteu spectrophotometric method. Quantification was carried out on the basis of a standard curve of gallic acid (10-100 mg/L). Results were expressed as mg gallic acid equivalents (mg GAE)/g dry leaf powder for the extract and as mg GAE/g dry extract for the biological experiment.

2.5 Experimental design

Thirty-six adult male albino rats weighing 188 ± 15.21 g were purchased from the Holding Company for Biological Products and Vaccines, Egypt. The rats were raised in the animal house in the Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt under laboratory conditions (relative humidity $85\pm 2\%$, temperature $22\pm 1^{\circ}\text{C}$ and 12 h light and 12 h dark cycle). They were fed on a basal diet and distilled water *ad-libitum* for one week for acclimatization after that they were weighed and randomly divided into two main groups: six rats were fed on a basal diet as a control (G1) and thirty rats for induction of hypocholesterolemia (total cholesterol ≥ 240 mg/dL) by feeding on hypercholesterolemic diet for 2 months according to the American Heart Association [20] and housed in individual cages. The animal study was carried out taking appropriate measures to minimize pain or discomfort taking all precautions regarding the care and use of animals for experimental procedures and with due clearance from the Institutional Animal Care and Use Committee (CU-IACUC, Cairo University). The study was approved by the Institute Animal Ethics Committee (CUIIF1116).

2.6 Diet composition

Basal diet, vitamin and mineral mixtures were prepared according to [21]. High fat diet (HFD) (1% cholesterol, 17% lard and 0.25% bile salts) was prepared according to [22].

The dry extract was reconstituted with normal saline as a vehicle before administration. Rats of Group 1 (G1) were fed on a basal diet (negative control) prepared according to [21]. Hypocholesterolemic rats were divided into the following groups: Rats of Group 2 (G2) were fed on a basal diet. Rats of Group 3 (G3) were fed on a basal diet and administered orally with a daily dose of moringa leaf extract (400 mg dry extract (31 mg GAE) /Kg bw). Rats of Group 4 (G4) were fed on a basal diet containing 0.737% moringa leaf powder (29.13 mg GAE/100 g diet). Rats of Group 5 (G5) were fed on a basal diet and orally administered with 2 mg fluvastatin/Kg bw. Rats of Group 6 (G6) (positive control) were continually fed on the high fat diet. Duration of the experiment was extended for 27 days. The animals had free access to both food and water throughout the duration of the study.

Blood samples were collected from retro-orbital plexus vein of all rats after fasted overnight (more than 12 h). Blood samples were kept for about half hour at room temperature before centrifugation at 3000 rpm for 10 min to separate serum for examination. The clear serum was separated and stored at -20°C for analysis, then the animals were anaesthetized and sacrificed and liver was quickly removed and washed with an ice cold saline to remove blood, dried between filter papers and weighed to calculate the relative weight percent.

2.7 Biochemical analyses of blood serum

Total cholesterol (TC) and Triglycerides (TG) were determined using kits obtained from DiaSys Diagnostic Systems GmbH, Holzheim, Germany. Low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) were determined using kits obtained from Biosystems S.A., Barcelona, Spain. Total lipids (TL) and malondialdehyde (MDA) were determined using kits from Bio-diagnostic, Giza, Egypt. Determinations were carried out according to manufacturer's instructions. The Atherogenic index was calculated according to [23] using the following formula

$$\text{Atherogenic index} = (\text{Total cholesterol} - \text{HDL-C}) / (\text{HDL-C})$$

2.8 Statistical Analyses

Chemical and biochemical determinations were carried out in triplicate. Results were expressed as mean \pm standard deviation (SD) and subjected to two-way analysis of variance (ANOVA). The data were analyzed using Duncan's Multiple Range Test. Statistical significance was determined at $P < 0.05$. Statistical analyses were performed using Costat-Statistics Software, version 6.4 (CoHort Software, California, USA).

III. Results And Discussion

The polyphenols content of the dry extract and dry leaf powder of *M. oleiferawas* 77.5 mg GAE/g dried extract and 39.53±0.30 mg GAE/g dried leaves, respectively. These results are in agreement with [6]. Results in Fig. 1 a. indicate that continual feeding of hypercholesterolemic rats (total cholesterol = 248.5±9.53 mg/dL) on a HFD (G6) for 27 days increased TC level to >290 mg/dL.

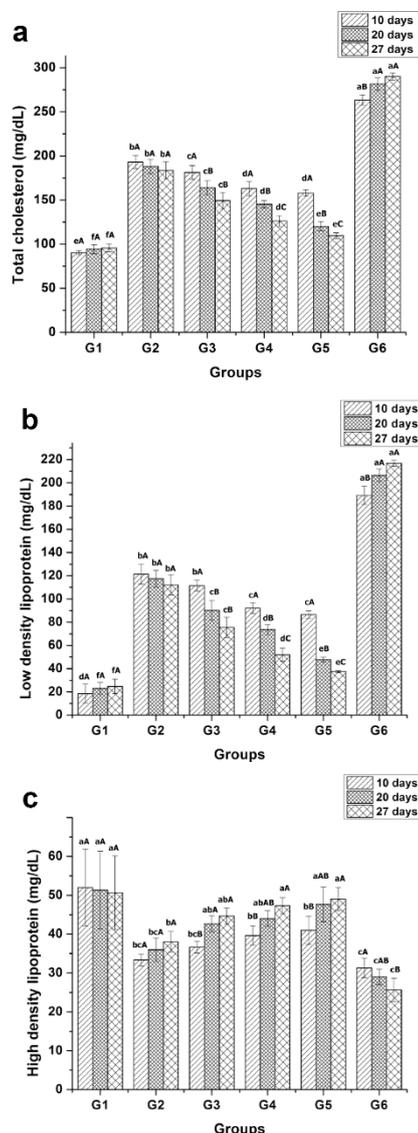


Fig.1 Total cholesterol, low density lipoprotein and high density lipoprotein of the experimental groups

Total cholesterol level of HCR fed on a basal diet with (G4) or without moringa powder (G2), treated with fluvastatin (G5), moringa extract (G3) was significantly lower than that of the rats of G6. These results are in agreement with [24]. They reported that leaf of *Moringaoleifera* is a potent hypocholesterolemic agent. Treating HCR with fluvastatin for 27 days lowered their TC to a level close to that of the control group (G1). A significant decrease in the TC level of rats of G4 and G5 (<127 mg/dL) was noticed after 27 days of the experiment.

Fig. 1 b. show that continual feeding hypercholesterolemic rats (HCR) on a HFD (G6) for 27 days increased the LDL level to >210 mg/dL. LDL-C is the primary target of therapy in patients with hypercholesterolemia. A high-risk LDL-C level was defined as a serum level of more than 160 mg/dL[25]. Free radicals induce by feeding on HFD during oxidation cause the peroxidation of lipids especially LDL thereby producing oxidized LDL. These oxidized LDL is taken up by the endothelial cells and macrophages and thus accelerates the atherosclerotic process [26]. Low density lipoprotein level of HCR fed on a basal diet with (G4) or without moringa powder (G2), treated with either fluvastatin (G5) or moringa extract (G3) was significantly lower than that of rats of G6. Treating HCR with fluvastatin for 27 days lowered their LDL to a level close to

that of the control group (G1). Extending duration of the experiment for 27 days was accompanied by a significant decrease in the LDL level of rats of groups G3-G5. Treating rats with moringa extract decreased lipid peroxidation and reduced LDL-cholesterol oxidation [27].

Fig. 1 c. indicate that continual feeding of hypercholesterolemic rats (HCR) on a HFD (G6) for 27 days decreased HDL level to <26 mg/dL. The highest level of HDL was recorded for G1 followed by G5, G4, and G3 at the end of the experiment. These results are in agreement with [28], they showed that dose of 400mg moringa leaf methanolic extract /kg bw increased the level of HDL of rats.

Feeding rats on the basal diet (G1) did not significantly increased their atherogenic index (AI) (<1) Fig. 2 a Feeding HCR(G6) on HFD for 27 days significantly increased the AI to >10. This level was almost 2-3 fold that found in the other investigated groups (G2-G5).

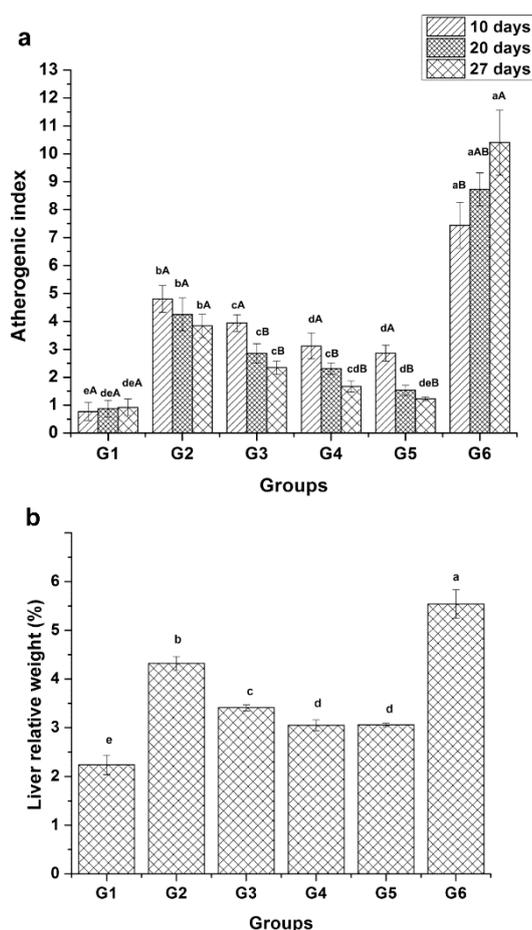


Fig.2 Atherogenic index and liver relative weight of the experimental groups

Atherogenic index indicates the disposition of foam cells or plaque or fatty infiltration or lipids in heart, coronary artery, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of the organs for the oxidative damage [29].

Feeding hypercholesterolemic rats on a basal diet containing moringa powder (G4) or orally administered with 2 mg fluvastatin/Kg bw (G5) for 27 days decreased significantly their AI to a level not significantly different than that of the control group. No further decrements in the AI was noticed in these groups when experiment was extended from 20 to 27 days. These results are in agreement with those found by [27]. Feeding HCR on a basal diet (G2) for 27 days did not significantly lowered their AI.

Results in Fig. 2 b indicate that the relative weight of liver of HCR that fed on a HFD for 27 days was significantly higher than that of the other investigated rats. No significant difference in the relative weight of liver could be noticed between HCR fed on a basal diet containing moringa leaf powder (G4) and those treated with fluvastatin (G5) for 27 days. The relative weight of liver of both groups was significantly close to that of the control group (G1). These results are in agreement with those found by [28]. They orally administered rats that were fed on HFD with moringa leaf methanolic extract and noticed a remarkable decrease in their organ's weight.

Continual feeding HCR on a HFD (G6) from 20 to 27days did not significantly increase their TG level(Fig. 3 a). Treating HCR with fluvastatin (G5) was significantly effective in reducing TG level followed by G4 that were fed on a basal diet containing moringa leaf powder (G4) for 27 days.

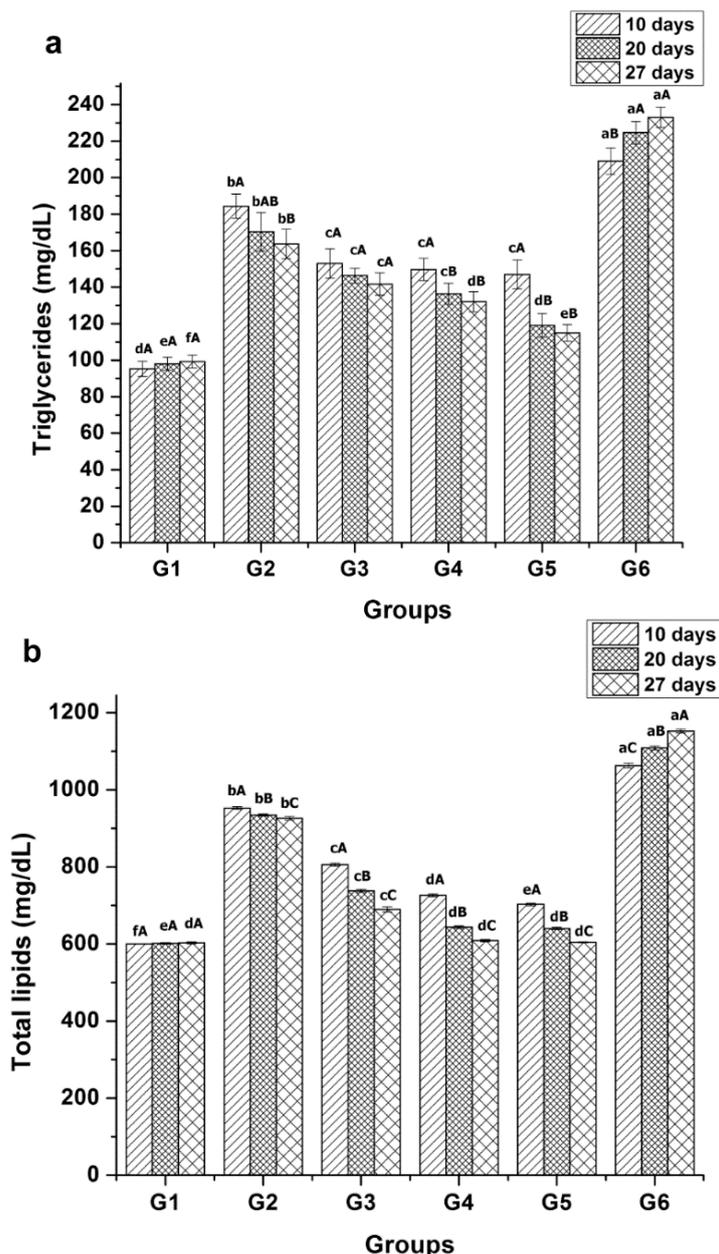


Fig.3 Triglycerides and total lipids of the experimental groups

Results in Fig. 3 b. indicate that continual feeding HCR on a HFD (G6) for 27 days significantly increased their TL level. Total lipid of HCR fed on a basal diet containing moringa powder (G4) or treated with fluvastatin (G5) for 27 days was insignificantly different than that of the control group (G1). Extending duration of the experiment for 27 days was accompanied by a significant decrease in the TL level of the rats of G2 and G3. Polyphenolic compounds possess a variety of biological activities, such as reduction of plasma lipids, which might be due to the up-regulation of LDL receptor expression [30].

Fig. 4 show that continual feeding HCR fed on a HFD (G6) for 27 days significantly increased their ALT(Fig. 4 a), AST (Fig. 4 b) and MDA (Fig. 4 c)levels.Feeding HCR on a basal diet containing moringa powder (G4) significantly decreased ALT and AST levels close to that of the control group (G1), followed by G3, G5 and G2, respectively.

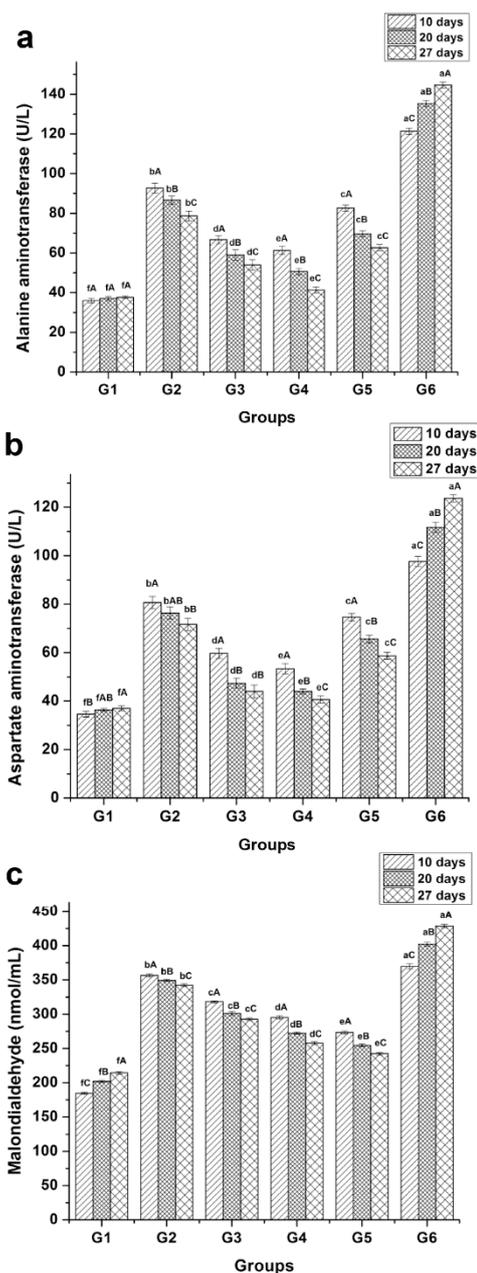


Fig.4 Alanine aminotransferase, aspartate aminotransferase and malondialdehyde of the experimental groups

Hypocholesterolemic rats feeding on a basal diet and treated with fluvastatin (G5) significantly decreased the MDA level close to that of the control group (G1), followed by G4, G3 and G2. High fat diet induced oxidative stress and lead to the generation of free radicals [26]. MDA is the end product of lipid peroxidation. Therefore, measurement of MDA gives an indirect evidence of LDL oxidation [27].

IV. Conclusion

Moringaoleifera leaf powder was superior than its extract as hypolipidemic and antioxidant agent. This superiority could be due to the fiber content in moringa leaves powder.

References

- [1]. M. Anandharaj, B. Sivasankari, and P. B. Rani, Effects of probiotics, prebiotics, and synbiotics on hypercholesterolemia: A review, *Chinese Journal of Biology*, 2014, Article ID 572754. doi.org/10.1155/2014/572754.
- [2]. B. A. Golomb, and M. A. Evans, Statin adverse effects: A review of the literature and evidence for a mitochondrial mechanism, *American Journal of Cardiovascular Drugs*, 8, 2008, 373-418.
- [3]. M. E. Abalaka, O. S. Olonitola, J. A. Onalapo, and H. I. Inabo, Evaluation of acute toxicity of *Momordica charantia* extract, using wistar rats to determine safety levels and usefulness of the plant in ethnochemotherapy, *International Journal of Applied Science*, 3, 2009, 1-6.

- [4]. WHO, Traditional Medicine Strategy 2002–2005. WHO, Geneva, 2002.
- [5]. M. L. Willcox, and G. Bodeker, Traditional herbal medicines for malaria, 2004, *BMJ* 329, 1156L 9, PMID: 15539672. doi:10.1136/bmj.329.7475.1156.
- [6]. S. Sreelatha, and P. R. Padma, Antioxidant activity and total phenolic content of *Moringaoleifera* leaves in two stages of maturity, *Plant Foods for Human Nutrition*, 64, 2009, 303–311.
- [7]. L. Gopalakrishnan, K. Doriya, and D. S. Kumar, *Moringaoleifera*: A review on nutritive importance and its medicinal application, *Food Science and Human Wellness*, 5, 2016, 49–56.
- [8]. S. M. Abdulkarim, K. Long, O. M. Lai, S. K. S. Muhammad, and H. M. Ghazali, Some physico-chemical properties of *Moringaoleifera* seed oil extracted using solvent and aqueous enzymatic methods, *Food Chemistry*, 93, 2005, 253–263.
- [9]. A. Sallau, S. Mada, and U. Ibrahim, Effect of boiling, simmering and blanching on the antinutritional content of *Moringaoleifera* leaves, *International Journal of Food Nutrition and Safety*, 2, 2012, 1-6.
- [10]. F. Anwar, S. Latif, M. Ashraf, and A. H. Gilani, *Moringaoleifera*: A food plant with multiple medicinal uses, *Phytotherapy Research*, 21, 2007, 17–25.
- [11]. J. L. Rockwood, B. G. Anderson, and D. A. Casamatta, Potential uses of *Moringaoleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations, *International Journal of Phototherapy Research*, 3, 2013, 61- 71.
- [12]. J. W. Fahey, *Moringaoleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. In: *Trees for Life Journal*, Part 1, 2005, 1-15.
- [13]. R. A. Ejoh, J. T. Dever, J. P. Mills, and S. A. Tanumihardjo, Small quantities of carotenoid-rich tropical green leafy vegetables indigenous to Africa maintain vitamin A status in Mongolian gerbils (*Merionesunguiculatus*), *British Journal of Nutrition*, 103, 2010, 1594-1601.
- [14]. V. S. Nambiar, and S. Seshadri, Bioavailability trials of β -carotene from fresh and dehydrated drumstick leaves (*Moringaoleifera*) in a rat model, *Plant Foods for Human Nutrition*, 56, 2001, 83-95.
- [15]. B. Moyo, P. J. Masika, A. Hugo, and V. Muchenje, Nutritional characterization of Moringa (*Moringaoleifera* Lam.) leaf, *African Journal of Biotechnology*, 10, 2011, 12925–12933.
- [16]. F. Alhakmani, S. Kumar, and S. A. Khan, Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringaoleifera*. *Asian Pacific Journal of Tropical Biomedicine*, 3, 2013, 623–627.
- [17]. B. Vongsak, P. Sithisarn, and W. Gritsanapan, Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringaoleifera* Lam., *Journal of Chromatographic Science*, 52, 2014, 641–645.
- [18]. J. Santas, R. Carbó, M. H. Gordon, and M. P. Almajano, Comparison of the antioxidant activity of two Spanish onion varieties, *Food Chemistry*, 107, 2008, 1210–1216.
- [19]. A. Arnous, D. P. Makris, and P. Kefalas, Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece, *Journal of Food Composition and Analysis*, 15, 2002, 655–665.
- [20]. P. A. Stapleton, A. G. Goodwill, M. E. James, R. W. Brock, and J. C. Frisbee, Hypercholesterolemia and microvascular dysfunction: interventional strategies, *Journal of Inflammation*, 7, 2010, 54.
- [21]. AOAC Official Methods of Analysis of AOAC International. 19th Ed., Association of Official Analytical Chemists, Gaithersburg, Maryland 20877-2417, USA, 2012.
- [22]. K. Srinivasan, B. Viswanad, L. Asrat, C. L. Kaul, and P. Ramarao, Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening, *Pharmacological Research*, 52, 2005, 313–320.
- [23]. F. Kayamori, and K. Igarashi, Effects of dietary nasunin on the serum cholesterol level in rats, *Bioscience, Biotechnology, and Biochemistry*, 58, 1994, 570–571.
- [24]. S. Ghasi, E. Nwobodo, and J. O. Ofili, Hypocholesterolemic effects of crude extract of leaf of *Moringaoleifera* Lam in high fat diet fed wistar rats, *Journal of Ethnopharmacology*, 69, 2000, 21-25.
- [25]. G. L. Vega, M. A. Denke, and S. M. Grundy, Metabolic basis of primary hypercholesterolemia, *Circulation*, 84, 1991, 118-128.
- [26]. N. P. Visavadiya, and A. V. R. L. Narasimhacharya, Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesterolemic rats, *Indian Journal of Pharmacology*, 37, 2005, 376-380.
- [27]. M. G. Rajanandh, M. N. Satishkumar, K. Elango, B. Suresh, *Moringaoleifera* Lam. A herbal medicine for hyperlipidemia: A preclinical report, *Asian Pacific Journal of Tropical Disease*, 2, 2012, S790-S795.
- [28]. S. Bais, S. G. Singh, and R. Sharma, Antiobesity and hypolipidemic activity of *Moringaoleifera* leaves against high fat diet-induced obesity in rats, *Advances in Biology*, 2014, Article ID 162914. doi.org/10.1155/2014/162914.
- [29]. J. K. Dabhi, J. K. Solanki, and A. Mehta, Antiatherosclerotic activity of ibuprofen, a non-selective COX inhibitor-an animal study, *Indian Journal of Experimental Biology*, 46, 2008, 476-81.
- [30]. D. J. Kuhn, A. C. Bums, A. Kazi, and Q. P. Dou, Direct inhibition of the ubiquitin-proteasome pathway by ester bond-containing green tea polyphenols is associated with increased expression of sterol regulatory element-binding protein 2 and LDL receptor, *Biochimica et Biophysica Acta*, 1682, 2004, 1–10.