A review on nickel nanoparticles as effective therapeutic agents for inflammation

Gangadhara Angajala, Subashini Radhakrishnan

Chemistry Research Laboratory, Organic Chemistry Division, School of Advanced Sciences, VIT University, 632014, Vellore

Correspondence: Subashini Radhakrishnan
E-mail: dr.subashini.r@gmail.com
Received: July 25, 2014
Published online: September 25, 2014

In recent years nanotechnology has emerged as one of the most recent active research fields in the area of science and technology. Nanotechnology possesses potential impact in solving many health related issues and extensive research has been carried out all over the world to explore its role in medicine. Development of cost-effective green processes for the synthesis of nanoparticles by using environmentally benign materials like plants and microorganisms offers huge benefits like biocompatibility and ecofriendliness especially in pharmaceutical and biomedical analysis. Due to their rich diversity and innate potential plants are considered as potential biofactories for the production of nanomaterials. This review highlights the antiinflammatory efficacy of nickel nanoparticles synthesized by using Aegle marmelos correa aqueous leaf extract. It also summarizes the role of β-sitosterol in exerting its antioxidant defence system during inflammation.

Keywords: Nickel nanoparticles; β-sitosterol; Anti-inflammatory; Atherosclerosis

To cite this article: Gangadhara Angajala, et al. A review on nickel nanoparticles as effective therapeutic agents for inflammation. Inflamm Cell Signal 2014; 1: e271. doi: 10.14800/ics.271.

Copyright: © 2014 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.

Introduction

Inflammation generally a normal response of living tissues to injury or irritant and it prepares the tissue for healing and repair. Generally, a body responds to injury of vascularized tissue with a sequence of events, collectively known as inflammation. The ultimate goal of inflammation is to replace injured tissue, and initiate the process of healing. Depending on the extent of injury, type of injury and the vascularity of tissue, inflammation may last from few minutes to few years. Inflammation may be caused by mechanical (either by pressure or foreign bodies), chemically (toxins), physically (temperature), by internal processes (uremia) and by microorganisms (bacteria, viruses, parasites) [1,2]. The process of inflammation is under the control of mast cells that are located closely near to the autonomic nerves. They are generally constituents of connective tissues with very bulky granules that possess mainly serotonin, heparin, histamine and bradykinin which were released from mast cells in response to injury, infection and by their degranulation. These constituents control almost all the inflammatory processes [3].

In some cases mast cells reacts under the influence of other controls for example they release serotonin in response to progesterone, and histamine in response to
estrogen. Another most important pathway in inflammation is arachidonic acid cascade mainly secured by eicosanoids which decrease the size and extent of tumor metastases in vivo [4]. Eicosanoids control mediators depending upon the genetic or by other factors. In extreme conditions inflammation may sometimes leads to mental disorders which is usually seen in chronic pain patients. In the degradation of tryptophan, indolamine 2,3 dioxygenase (IDO) is considered as a rate limiting enzyme and mainly induced in wide variety of cells during inflammation by cytokines. Increased IDO enhance tryptophan degradation and subsequent serotonin depletion which simultaneously associated with mental depression [5].

The interaction of the cellular immune system with exogenous or endogenous antigens results in the generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species) leading to signalling cascades that generate the release of proinflammatory cytokines and chemokines. Cytokines are soluble mediators of intracellular communications that are directly linked to the ion channels regulates, haemopoiesis, specific and non-specific immune responses and tissue repair. Transmission of extracellular information in to the cytoplasm and nucleus is triggered by the binding of cytokines to their specific receptors. The information is transmitted by various signalling pathways like mitogen-activated protein kinase (MAPK) and nuclear factor κB [6]. Sustained vigorous inflammation can sometimes lead to cellular damage or hyperplasia which subsequently leads to the overproduction of reactive oxygen species (ROS) from inflammatory cells. A wide variety of cytokines induces the production of ROS in nonphagocytic cells during inflammatory process, by specifically binding to receptors. Interaction of ROS with DNA especially in mitotic cells leads to permanent genomic mutations. During inflammation cellular antioxidant systems activate genes involved in DNA repair and thereby respond to free radical overproduction. The rate of ROS induced DNA damage is more during chronic inflammation and this process substantially causes depletion of cellular antioxidants [7].

Inflammatory process is either acute or chronic. When acute, it occurs as an immediate response to trauma whereas in case of chronic, it usually reflects ongoing response to a longer term medical condition such as arthritis. Generally inflammation is completely different to that of infection, as infection is normally caused by various microorganisms, which sometimes provoke inflammation, however both infection and inflammation are treated separately [8]. In olden days inflammation was characterized by visual observations such as redness, swelling, pain, heat and loss of function. In this modern era, although inflammation was viewed as a part of the healing process, before the end of 19th century inflammation was treated as an undesirable harmful response to the host [9].

Today inflammation is considered as far more complex and a multidisciplinary approach is needed in order to understand molecular mechanism of inflammation thoroughly. Classically to study the processes involved in maintaining and initiating inflammatory conditions a sound knowledge on immune system is prerequisite. But today it is well recognized that the underlying genetics and molecular biology to basic cellular responses also play a key role in understanding and identifying genetic predisposition to various inflammatory mediated sequences [10]. On the other hand pharmacological studies are quite necessary to identify targets and develop novel therapeutic agents to bring relief from chronic life-threatening inflammatory diseases. Now a day the role of inflammation as healing, restorative process as well as its aggressive role is also more widely understood. The understanding of molecular and cellular pathways implicated in the process of inflammation has increased dramatically over the recent years and this laid foundation to the discovery of many therapeutic novel lead compounds for the development of drugs in the treatment of chronic inflammatory diseases [11]. The continuous advent of new findings in the field of molecular genetics, biochemical, immune histochemical in association with functional animal models together with the
clinical research have drastically enhanced the curiosity to focus on various mechanisms underlying the overall process of inflammation.

For many years pharmaceutical industries attempted to develop various NSAIDS (NonSteroidal Antiinflammatory Drugs) with reduced side effects to produce gastric ulcers with the demonstration that clinically used NSAIDS inhibits the cyclooxygenase enzyme which is also located in the gastric mucosa. The differential responsiveness of individual to various therapeutic drugs and initiation of some inflammatory response in some patients with asthma by aspirin has led to the concept of pharmacogenomics with a goal of extending therapy tailored to the individual. In similar approach the usage of glucocorticoids to treat inflammation had increased dramatically over the past few years. Unlike the NSAIDS they do not relieve pain but inhibit leucocyte function thereby reducing inflammation. Large number of patients suffering from severe chronic inflammation fails to respond to topical therapy or conventional systemic therapy which results in enormous socio-economic burdens underlies the necessity to develop modern novel therapies. Thus there is a clear unmet medical emergency for an effective therapeutic agent that gives relief from the symptoms of inflammation systematically.\textsuperscript{[12-14]}

Over the past decades plants have been considered as basis for traditional medical system throughout the world for many years. Most of the secondary metabolites obtained have led to the development of innovative drugs. Over the past decade, identification and isolation of plant derived substances for the treatment of various ailments has increased dramatically. Various medicinal plants have been in use for the treatment of various inflammatory diseases. Even though the recovery is slow, the immense therapeutic use of herbs is becoming popular because of its inability to cause side effects.\textsuperscript{[15]} Now a day the search for the development of therapeutic anti-inflammatory drugs from the array of traditional herbal medicine is intensifying. An extensive systemic research has been carried out by pharmacologists from all over the world to explore potential phytoconstituents from medicinal plants for the development of products for their better economic and therapeutic utilization.\textsuperscript{[16]}

Drugs derived from natural sources can modulate various inflammatory mediators (protein kinases, calcium, cGMP and cAMP), the expression of proinflammatory molecules such as cytokines (IL-1\textbeta, TNF-\alpha), cyclooxygenase (COX-2), inducible NO synthase (iNOS), neuropeptides and proteases and the expression of key transcription factors such as NF-\textkappa\textbeta, AP-1, and
protooncogens (c-jun, c-fos, c-myc)\textsuperscript{[17, 18]}. Development of drugs that can be used as effective agents in the treatment of various inflammatory diseases play a key role in overall health of the humans. Development of drugs from plant source involves multidisciplinary approach combining phytochemical, botanical, ethnobotanical and biological techniques\textsuperscript{[19]}. Most of the natural products derived from plants are in clinical use while some drugs undergoing phase II and phase III clinical trials. So the current review highlights the antiinflammatory efficacy of nickel nanoparticles biofabricated from aqueous leaf extract of Aegle marmelos Correa.

Aegle marmelos Correa (AmC) commonly belongs to the family rutaceae and it is medium sized plant originates from India and presently distributed all over the country. In India it grows throughout the outer Himalayan regions, deciduous forests South Indian plateau with altitudes ranging from 210 to 1260 m. The fruit possess good pharmacological properties especially in the treatment of dysentery and chronic diarrhea; it also acts as tonic for heart and brain. The Bael fruit is one of the most nutritious edible fruits, rich in carotenoids, riboflavin and pectin which is used for the preparation of various products like candy, squash, toffee, slab, pulp powder and nectar\textsuperscript{[20]}.

AmC belongs to monotypic genera of sub family Aurantioidae, tribe Clauseneae and sub tribe Balsamocitrinae and family Rutaceae. It is considered as herbal medicine for the treatment of various ailments. Every part of this plant has its own medicinal property and it is generally used for the treatment of diarrhea, dyspepsia, dysentery, mental diseases, diabetes, jaundice, antifungal, anti-inflammatory, antipyretic, analgesic, antiproliferative, antidiabetic, cardiotonic, regeneration of damaged pancreas, antiviral, antiulcer, anticancer, hemolytic, larvicidal, hepatoprotective and antibacterial activity asthma, anaemia, healing of wounds, fractures, swollen joints, and also for the management of diabetes\textsuperscript{[21-24]}. Various chemical constituents like alkaloids, coumarins, steroids are identified from different parts of the plant\textsuperscript{[25, 26]}.

In recent years the use of nanotechnology in medicine dramatically increased all over the world especially in drug delivery. Research was being carried in using nanoparticles as therapeutic drugs and as well as drug carriers in order to reduce the toxicity and side effects associated with chemical drugs\textsuperscript{[27]}. Because the kind of hazards imposed by nanoparticles are beyond the reach of conventional hazards caused by chemical classical delivery systems. Nanoparticles being small in size have the potential to cross blood-brain barrier and elicit its biological effect. A multitude of substances are still under investigation for the preparation of nanoparticles which have the ability to interact deep inside the tissues and cells and exert its pharmacological effect. For pharmaceutical industry the current parameters seems to be adequate to explore the potential therapeutic applications of nanoparticles in medicine\textsuperscript{[27-29]}.

Nickel is considered to be an essential element in plants,
animals and microorganisms. Nickel plays a key role in the reduction of carbon monoxide to acetate in acetogenic bacteria by activating carbon monoxide dehydrogenase. Nickel interacts with iron in haemoglobin and helps in the transport of oxygen in addition to this it also stimulates metabolism. It helps in the transmission of genetic code (DNA and RNA) and also present in enzyme matrix of certain metabolize sugars. Nickel also helps in nerve impulse, muscle excitation and contraction by substituting calcium in the excitation process and simultaneously involves in the binding with membrane ligands such as phosphate groups of phospholipids. Nickel deficiency in rats led to reduced iron levels in organs, decrease in haemoglobin which further leads to anaemia. Nickel also serves as cofactor in the activation of calcineurin a calmodulin dependent phosphoprotein phosphatases. It also controls the formation of cGMP (cyclic guanosine monophosphate) a cyclic nucleotide that regulates the signals various physiological processes such as sodium metabolism, high blood pressure control, cardiovascular health, sperm physiology. Consistently, nickel is also present in RNA and commonly bound to amino acids, proteins and serum albumins [30-32].

In the present review we are reporting the phytofabrication of NiNPs using aqueous leaf extracts of AmC. In continuation to this we also made sincere attempt to explore the mechanistic pathway involved in the synergistic efficacy of β-sitosterol and nickel nanoparticles in inducing anti-inflammation.

### Synthesis of nickel nanoparticles

Aqueous solution (1 mM) of NiCl₂ was freshly prepared with milli Q water and used for the synthesis of nickel nanoparticles (NiNPs) of AmC. 10 ml of aqueous extract was added to nearly 80 ml of 1 mM NiCl₂ solution. The effect of temperature on the synthesis of copper NPs was monitored both at room temperature 30ºC for 24 h and at 60 ºC on water bath for 4 h [33][Fig.1].

### Characterization of nickel nanoparticles

The plant mediated reduction of Ni²⁺ ion in solution was monitored by using UV-vis spectrophotometer (Jasco, model no: V-670), FT-IR spectroscopy (Shimadzu, model no: IR affinity-1), GC-MS (Perkin Elmer: clarus 680/600).

Further characterization of nanoparticles was done using SEM analysis (Scanning electron microscopy, Tescan Vega 3 Sbu) [33] and AFM (Atomic Force Microscopy, Nanosurf Easy Scan 2, Version 1.3). XRD analysis (Advance Powder X-ray diffract meter, Bruker, Germany, model no: D8) and TGA (Thermo gravimetric analysis, Schimadzu, Model No: DT-50).

### Results

#### In-vitro antiinflammatory efficacy

_In-vitro_ anti-inflammatory studies of synthesized NiNPs of AmC (Fig.2) was carried out and compared with different leaf extracts of AmC by two methods such as membrane stabilization test and albumin denaturation assay as per reported method [33,36]. NiNPs showed good anti-inflammatory activity which was comparable to that of standard Aceclofenac.

#### GC-MS analysis of Aegle marmelos Correa leaf extracts

The chemical constituents of leaf extracts of AmC were...
analyzed by using Perkin Elmer GC-MS (clarus 680/600) having 5973 N mass selective detector equipped with split or split less capillary injection port (30.0 m × 240 µm).

**Chemical Constituents of Aegle marmelos Correa leaf and fruit extracts**

A total of 20 compounds were identified from GC-MS analysis representing 98.20% of total composition of leaf extract. The major compounds identified include β-sitosterol (55.65 %), camphene (5.67 %), α-phellandrene and phenylethylcinnamid (4.01 %). The percentage composition of the remaining compounds ranged from 0.31 % to 3.75 % [33].

**Discussion**

**Molecular mechanism involved in the antiinflammatory efficacy of NiNPs**

The β-sitosterol identified from aqueous leaf extract of AmC is a plant sterol which has structural similarity with cholesterol. It is considered as an important dietary constituent which is hydrophobic and considered as good biomarker because of its biological effectiveness. It is used in the treatment of prostate enlargement in order to boost the function of T-cells and drives to operate and function more efficiently. β-sitosterol inhibits cancer cell proliferation by decreasing the expression of PCNA or β-catenin and simultaneously activating the Fas signaling. In vitro toxicological studies also showed that the administration of β-sitosterol was safe and nontoxic. β-sitosterol along with its glycoside β-sitosteroline helps in normalizing T-cell function, DHEA: cortisol ratio and dampening over active responses by restoring optimum immune parameters. β-sitosterol also capable of reducing the secretion of proinflammatory cytokines and tumour necrosis factor-alpha in addition to this it also reduce experimentally induced edema. β-sitosterol also prevents the growth of specific type of tumor cells in vitro and exerts its impact on tumor necrosis factor-α (TNF- α) relating signal in HAEC such as phosphorylation and activation of nuclear factor-κB (NFκB). β-sitosterol initiates apoptotic induced programmed cell death pathway in human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [34-42].

β-sitosterol possess anti-inflammatory efficacy by inhibiting cholesterol absorption in the lower intestine thereby reducing excess of cholesterol in the blood preventing atherosclerosis. Atherosclerosis is a chronic inflammatory disease commonly associated with adhesion of vascular endothelium, increased oxidative stress and subsequent migration into the cells. The over expression of adhesion molecules, by endothelial cells leads to oxidative modification of low density lipoprotein (LDL), endothelium dysfunction and artherosclerotic lesions [29-30]. β- sitosterol mainly associated with cardiovascular protection in artherosclerosis by mainly exerting its antioxidant defensive system, preventing the over expression of vascular, intracellular adhesion molecules and the subsequent attachment of monocytes in human aortic endothelial cells (HAECs). β-sitosterol effectively boosts the function of T-cells and immune system to function and operate efficiently thereby it prevents rheumatoid arthritis and its associated autoimmune diseases [43-51].

**Role of high surface area-to-volume ratio in inducing therapeutic effect**

Nickel nanoparticles are special and interesting because their physical and chemical properties are different when compared to their macro counter parts. Due to their small size nickel nanoparticles possess unique properties; regardless of their chemical properties have high surface to volume ratios that are extremely high. This automatically causes their physical properties to be dominated by the effect of capping agents and surface atoms on the surface of nickel nanoparticles. The high surface area-to-volume ratio of nickel nanocrystals can sometimes leads to unexpected properties. A particle with high surface area possesses many reaction sites than a particle with low surface area and thus leads to higher chemical reactivity. High surface area-to-volume ratios have interesting applications such as increased therapeutic efficacy and in catalysis. One biological example is body’s digestive system, where within the small intestine there are millions of folds and sub folds that increase the surface area of inner lining of the digestive system [52].

The synergistic efficacy of both β-sitosterol and nickel induces an enhanced anti-inflammatory efficacy. [Fig.3] This effect was mainly due to increase in surface area to volume ratio which automatically increases the dominance behaviour of atoms on the surface rather than the interior of the particle. This leads to an increase in the surface energy which is directly proportional to its biological effectiveness. Increase in surface area of NiNPs makes maximum interaction of β-sitosterol with the receptor site by producing its therapeutic effect. It generally decreases the systemic inflammation and simultaneously supports proliferation of pheripheral blood lymphocytes thereby enhancing the cytotoxic efficacy of natural killer cells. NiNPs of Aegle marmelos Correa accompanied by β-sitosterol actively triggered by oxidative stress during inflammation which automatically eliminates free-radical oxidants in extravascular region via H-atom donation (Fig.4). As the surface area of NiNPs of AmC is higher than that of aqueous leaf extract predominantly it shows better anti-inflammatory activity which was on par to that of standard Aceclofenac. This efficacy is mainly attributed to β-sitosterol of AmC surrounding the NiNPs.
Conclusions

In the field of nanotechnology there is an effective need to develop cost-effective, reliable, green processes for the synthesis of metal nanoparticles by using environmentally benign biomaterials like plants and microorganisms. Here, we have reviewed the biosynthesis of NiNps from AmC aqueous leaf extract having triangle shape with face centered cubic structure showing an average particle size of 50-100 nm. This paper summarizes the green production of nickel nanoparticles from AmC aqueous leaf extract and its synergistic efficacy with β-sitosterol to induce antiinflammation. Both extracellular scavenging efficacy and intracellular inhibitory effect of nickel nanoparticles has been reviewed. Further in future, extensive research has to be carried out fully to explore the role of nickel nanoparticles and β-sitosterol as an effective therapeutic agent for chronic inflammation and its associated symptoms.

Acknowledgements

Authors thank VIT University for providing the facilities to successfully complete this review in a paper form.

Conflicting interests

The authors have declared that no competing interests exist.

References

1. Hold GL, El-Omar ME. Genetic aspects of inflammation and cancer. Biochem J 2008; 410:225-235.
2. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420:860-97.
3. Theoharides TC, Kalogeromitros. The critical role of mast cells in allergy and inflammation. Ann NY Acad Sci 2006; 1088: 78-99.
4. Funk CD. Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology. Science 2001; 294:1871-1875.
5. Muller N. The role of antiinflammatory treatment in psychiatric disorders. Psychiatric Danubina 2013; 25: 292-298.
6. Yang D, Elner SG, Bian ZM, Till GO, Petty HR, Elner VM. Proinflammatory cytokines increase reactive oxygen species through mitochondria and NADH oxidase in cultured RPE cells. Experimental Eye Research 2007; 85:462-472.
7. Mittwoch-Jaffe T, Shalit F, Srendi B, Yehuda S. Modification of cytokine secretion following mild emotional stimuli. Neuroreport 1995; 6:79-792.
8. Conner EM, Grisham MB. Inflammation, free radicals and antioxidants. Nutrition 1996; 12 : 274-277.
9. Klauing JE, Kamendulis LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004; 44: 239-267.
10. Gulumain M. The role of oxidative stress is diseases caused by mineral dusts and fibres: current status and future of prophylaxis and treatment. Mol cell Biochem 1999; 196:69-77.
11. Maes M. Evidence for an immune response in major depression a review and hypothesis. Prog NeuroPschoPharmacol Biol Psychiatry 1995; 19: 11-38.
12. Mccarthy DM. Comparative toxicity of non-steroidal antiinflammatory drugs. Am J Med 1999; 107:378-478.
13. Richy F, Bruyere O, Ethgen O, Rabenda V, Bouvenot G, Audran M, et al. Time dependent risk of GI complications induced by non steroid antiinflammatory drug use: a consensus statement using a meta-analytic approach. Ann Rheum Dis 2004; 63:759-766.
14. FlizGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase 2. New Engl J Med 2001; 345: 433-442.
15. Agarwal VS. Rural economics of medicinal plant : Vegetation in the forest. Drug plants of India. Kalyani Publishers India 1997; 1:1-160.
16. Kirthikar KR, Basu BD. Indian medicinal plants. International Book Distributors, Dehra Dun 1975:212-213.
17. Dinarello CA. Interleukin-1 beta, interleukin-18, and interleukin-1 beta converting enzyme. Ann NY Acad Sci 1998; 856:1-11.
18. Border WA, Noble NA. Mechanisms of disease. Transforming growth factor β in tissue fibrosis. N Engl J Med 1994; 331:1286-1292.
19. Adams DH, Lloyd AR. Chemokines: leukocyte recruit and activation cytokines. Lancet 1997; 349: 490-495.
20. Dhankar S, Ruhl S, Balhara M, Chhillar AK. Aegle marmelos (Linn.) Correa: A potential source of Phytomedicine. J Med Plants Res 2011; 5:1497-1507.
21. Hema CG, Lalitha KK. Screening of pharmacological actions of Aegle marmelos correa. Indian J Pharm 1999; 20: 80-85.
22. Balakumar S, Rajan S, Thirunalasundar T, Jeevas. Antifungal activity of Aegle marmelos correa leaf extracts on dermatophytes. Asian Pacific Journal of Tropical Biomedicine 2011; 1: 309-312.
23. Arul V, Miyazaki S, Dananjayan R. Studies on the antiinflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos correa. J Ethnopharmacol 2005; 96: 159-163.
24. Maity P, Hansda D, Bandypadhyay U. Biological activities of crude extracts and chemical constituents of Beal, Aegle marmelos (L) corr. Indian J Exp Biol 2009; 47: 849-861.
25. Haravey SK. A preliminary communication on the action of Aegle marmelos (Bael) on heart. Indian J Med Res 1968; 56: 327-331.
26. Gupta D, John P, Pankaj KR, Koushik R, Yadav R. Pharmacological review of Aegle marmelos cor. Fruits. International Journal of Pharmaceutical Sciences and Research 2011; 2: 2031-2036
27. Parveen S, Misra S, Sahoo SK. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomed-Nanotechnol 2012; 8: 147-166.
28. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances 2013; 31: 346-356.

29. Thakkar KN, Mhatre SS, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomedicine 2010; 6: 257-262.

30. Cemple M, Nickel G. Nickel: A Review of Its Sources and Environmental Toxicology. Polish J of Environ Stud 2006; 15: 375-382.

31. Chohan ZH, Sherazi SKA. Biological Role of Cobalt (II), Copper (II) and Nickel (II) Metal Ions on the Antibacterial Properties of Some Nicotinoyl-Hydrazine Derived Compounds. Met Based Drugs 1997; 4: 69-74.

32. Thauer RK, Diekert G, Schonheit P. Biological role of nickel. Trends in Biochemical sciences 1980; 5: 304-306.

33. Angajala G, Ramya R, Subashini R. In-vitro antiinflammatory and mosquito larvicidal efficacy of nickel nanoparticles phytofabricated from aqueous leaf extracts of Aegle marmelos Correa. Acta Tropica 2014; 135: 19-26.

34. St-Onge MP, Jones PJ. Phytosterols and human lipid metabolism: efficacy, safety and novel foods. Lipids 2003; 38: 367-375.

35. Katan MB, Grundy SM, Jones P, Law M. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003; 78: 965-978.

36. Nashed B, Yeganeb H, HayGlass KT, Moghadasi MH. Antiatherogenic effects of dietary plant sterols are associated with inhibition of proinflammatory cytokine production in Apo E-KO mice. J Nutr 2005; 135: 2438-2444.

37. Bustos P, Duffau C, Pacheco C, Ulloa N. Beta-sitosterol modulation of monocytes-endothelial cell interaction: A comparison to female hormones. Maturitas 2008; 60: 202-208.

38. Wolle J, Hill R, Ferguson E, Devall LJ. Selective inhibition of tumor necrosis factor-induced vascular cell adhesion molecule-1 gene expression by a novel flavonoid. Lack of effect on transcription factor NF-kappa B. Arterioscler Thromb Vasc Biol 1996; 16: 1501-1508.

39. Marinangeli CP, Varady KA, Jones PJ. Plant sterols combined with exercise for the treatment of hypercholesterolemia.