IJPSR (2012), Vol. 3, Issue 07 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 February, 2012; received in revised form 29 June, 2012; accepted 13 July, 2012

INHIBITION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG Co-A) REDUCTASE IN LIVER MICROSOMES BY *MORINGA OLEIFERA* L. POLYPHENOLS

Vanitha Reddy P. [1], Faiyaz Ahmed [2] and Asna Urooj*[1]

Department of Studies in Food Science and Nutrition, University of Mysore [1], Manasagangotri, Mysore- 570006, India

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia [2], 11800 Minden, Penang, Malaysia

Keywords:
Moringa oleifera,
Polyphenols,
Antioxidants,
Cholesterol,
HMG CoA Reductase,
Coenzyme A

Correspondence to Author:

Dr. Asna Urooj

Associate Professor, Department of Studies in Food Science and Nutrition, University of Mysore, Manasagangotri, Mysore 570006, India

E-mail: asnaurooj@foodsci.unimysore.ac.in

ABSTRACT

Phytochemicals are the bioactive components present at micro level in our daily diet, especially the phenolic antioxidants, contribute to the prevention of cardiovascular and other degenerative diseases. *Moringa oleifera* (MO) leaves polyphenols effect on the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase was studied using a spectrophotometric assay based on the stoichiometric formation of coenzyme A (SH) during the reduction of microsomal HMG CoA to mevalonate at different concentrations (200- 400 µg). Activity of HMG- CoA reductase was inhibited by MO polyphenols significantly ($p<0.05$) at the concentrations studied. The Coenzyme A released at the end of assay in the control was higher (18.87±0.12 n Moles) significantly than the MO polyphenol extract, which was dose dependent (14.69±0.07 to 10.26±0.33 n Moles). The results reveal the potency of the MO polyphenols in inhibiting the HMG- CoA reductase. Hence, the MO polyphenols can be explored for its role as a cholesterol lowering agent.

INTRODUCTION: Elevated plasma cholesterol concentration is a well known risk factor for the development of atherosclerosis and coronary heart disease (CHD). Consequently, public health recommendations are aimed at reducing plasma cholesterol concentration [1].

In humans, most cholesterol biosynthesis occurs in the liver. The enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase; E.C 1.1.1.34) is responsible for catalysing the NADPH-dependent, two-step reduction of HMG-CoA to mevalonate. This reaction is a highly regulated process within the cholesterol biosynthetic pathway, and so is an attractive target for intervention in the treatment of hypercholesterolaemia. HMG CoA Reductase is found in cytosol and microsomal fractions of the cell [2].

Inhibition of HMG CoA reductase prevents and reverses MYC-induced lymphomagenesis, treats adenocarcinomas of the prostate, stomach, lung, breast and colon and Ewing's sarcomas, promotes anti-inflammatory pathways in vascular wall [3] and inhibits cholesterol biosynthesis [2]. Several inhibitors of HMG-CoA reductase have been developed as cholesterol-lowering therapeutic agents known as statins, fibrates, niacin etc [4].

Medicinal plants and their extracts have gained increasing importance over the past years, since they can be used as sources of herbal drugs [5].

A number of plants with potent therapeutic components such as fibers, sterols, saponins, polyphenols, flavonoids, etc., have been investigated for their antihyperlipidemic, antioxidant, phytohormones, antiatherosclerotic and enzyme modulating properties. These compounds are reported to be beneficial with great variation in magnitude and mechanism of action and hence, have a potential therapeutic value in combating multifactorial atherosclerotic disorders [6].

Research strongly suggests that protection against degenerative diseases requires the right balance of a multitude of phytoprotectants including essential fatty acids, vitamins, antioxidants and dietary fiber components [7]. Exploration of the chemical constituents of the plants and pharmacological screening will thus provide the basis for developing new life saving drugs and functional foods. In more recent years, polyphenols have received much attention in disease treatment and prevention due to their *in-vivo* and *in-vitro* antioxidant capabilities.

Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of CVD [8]. Many plants with medicinal value have also been screened for their antioxidant activity by *in-vitro* and *ex-vivo* with different substrates, the main aim of the studies is to explore the potency of the selected plant against oxidation and free radical generation or scavenging.

The leaves of *Moringa oleifera* Lam (Moringaceae) are used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients [9]. The scientific basis for their use in hypercholesterolemia has been reported. Oral administration of *Moringa oleifera* extracts which contain the alkaloïd moringine, identical to benzylamine, has also been shown to prevent hyperglycemia in alloxan-induced diabetic rats [10].

The hypocholesterolemic effect of crude extract of *Moringa oleifera* leaf has been studied in animal species (9). Results of a study conducted in our laboratory showed reduction in cholesterol levels in rats on oral supplementation of MO leaves powder [11]. In addition, we have explored the antioxidant activity, stability and use of *Moringa* in food systems [12, 13, 14, 15].

With this background, an attempt was made to determine the potency of the polyphenols from *Moringa Oleifera* leaves on the inhibition of the HMG CoA reductase activity, it is hoped that this information will help in understanding the mechanism and role of selected sample in modulating the cholesterol metabolism.

MATERIALS AND METHODS:

Chemicals: All the chemicals were of analytical grade. The substrate HMG CoA, dithiothreitol, triethanol-amine and NADPH were obtained from Sigma Aldrich Chemicals, Bangalore.

Plant Material: The selected plant material, *Moringa oleifera* (MO) leaves were collected locally, washed and dried in a hot air oven at 55°C. Dry leaves were ground separately and passed through a 60 mesh sieve and kept in air tight containers at 4°C until further use.

Preparation of Polyphenol Extract: Different solvent extracts were prepared from the MO leaves and among all the 80% Methanol extract found to be richer in polyphenols [13].

Extraction: A 15g sample was extracted with 100ml solvent, (methanol: water, 8: 2) in a mechanical shaker. The extracts were evaporated at 40º C under reduced pressure to dryness in a rotary evaporator (Superfit, India). The extract was rich in chlorophyll and to avoid the interference of the green color during the analysis the chlorophyll was separated by the method given by Alexis Rich and Christine [16], briefly, hexane was added to the 80% methanol extract and shaken for 30 minutes , as chlorophyll is readily soluble in hexane, a chlorophyll rich hexane layer is formed on the top of the extract leaving the other polar components especially phenolic compounds in the water and methanol layer and this polyphenol (30-40%) rich extract was further dried and stored in air-tight container at 0^{o}C until used.

Animal and liver: A healthy male adult rat was fasted 24hr and sacrificed after 9:00 pm to obtain the active HMG CoA reductase enzyme.

Preparation of Microsomes: The procedure described here is partially modified from that reported by Shapiro and Rodwell, 1971 [17]. The liver was

immediately removed and placed in cold buffer (0-4°C) at pH 7.4. The buffer contained 0.1 M triethanolamine. HCl, 0.02 M EDTA, and 2.0mM dithiothreitol. The liver was thoroughly chilled, blotted, and weighed. The tissue was minced with scissors and homogenized with 3.5 ml of buffer/g of tissue. The homogenization was done with six strokes of a smooth-walled, glass Remi homogenizer with a loose-fitting Teflon pestle (0.5 mm radial clearance). The homogenate was centrifuged for 10 min at 12,000 g to remove cell debris and mitochondria.

After the supernatant solution was carefully removed to prevent contamination with mitochondria and recentrifuged for 10 min at 12,000 g to ensure removal of mitochondria, it was then centrifuged at 60,000 g for 60 min. The 60,000g microsomal pellet was then rinsed with buffer and frozen in a freezer (-20°C). Frozen microsomes were resuspended in 0.1 M triethanolamine buffer, pH 7.4, containing 0.02M EDTA and 10mM dithiothreitol and allowed to stand 60 min packed in ice; they were then centrifuged at 60,000g for 45 min. The resuspended microsomes to be used for the assay were diluted with buffer to give a protein concentration of 5-10 mg/ml.

3-Hydroxy-3-methylglutaryl CoA Reductase Assay as per Frankel H, Hulcher and Wayne H Oleson, 1973 [18]. For the assay of 3-hydroxy-3-methylglutaryl CoA reductase, the incubation mixtures normally contain 0.5-1 .0 mg of microsomal protein, 150 nmoles of HMG CoA, and either 2 µmoles of NADPH or, preferably, 2 µmoles of NADP, 2 units of glucose-6-phosphate dehydrogenase, and 3 µmoles of glucose-6-phosphate. These components are added to 0.8 ml of 0.1 M triethanolamine-0.02 M EDTA buffer at pH 7.4 without dithiothreitol.

The dithiothreitol (0.2 µmole) was added along with the microsomal preparation. The final incubation volume was 1 ml. Control tubes were as follows: *(a*) All components, (b) all components except NADPH, (C) all components except NADPH with 200to 400µg of Polyphenols extract. The reaction is started by the addition of microsomes, and the mixture is incubated in Corex centrifuge tubes at 37°C for 30 min. After incubation, 20µl of 1 X M sodium arsenite solution is added to facilitate removal of soluble protein, and after 1 min the reaction is terminated by the addition of 0.1 ml of 2.0 M citrate buffer, pH 3.5**,** containing 3% sodium tungstate to give a final pH of 4.0 to precipitate the microsomal protein. After 10 min in a 37°C water bath, the tubes are centrifuged at 25,000 g for 15 min to remove protein.

1 ml of the supernatant is transferred to a tube with stopper, and just before assay the pH is brought to 8.0 by addition of 0.2 ml of 2 M Tris buffer, pH 10.6, and 0.1 ml of 2 M Tris buffer, pH 8.0. The formation of the dithiol-arsenite complex is complete in 3-4 min after the addition of 50 µl of 0.4 M sodium arsenite. The concentration of monothiol is determined by reacting DTNB with the reaction mixture in a cuvette placed in a recording spectrophotometer. 20 µl of 3 mM DTNB in 0.1 M triethanolamine 42 M EDTA buffer, pH 7.4, is added to 1.0 ml of the solution in a 1.0-ml cuvette, and the time is indicated on the recorder. The contents are mixed and the absorbance is measured for 4 min at 412 nm.

The monothiol reacts immediately but the dithiol reacts slowly, giving a linear increase in absorbance with time. The absorbance due to monothiol is determined by extrapolating the linear portion of the curve back to the time of addition of DTNB. The concentration of monothiol is calculated using the molar extinction coefficient of 1.36 X lo4 (Ellman GL,. 1959). The conversion to nanoMoles of CoA formed per minute is as follows:

$$\text{n moles/min} = \frac{[\text{A reaction} - \text{A control}] \times 1.43}{0.0136 \times \text{time}}$$

The value of 1.43 is the dilution factor, and A is the absorbance at 412 nm. The difference in absorbance between the complete reaction and that of all components except NADPH represents the activity due to HMG CoA reductase.

Statistical Analysis: Each experiment was conducted in 3 replicates and data are expressed as Mean$\pm$ SD. The HMG CoA reductase inhibition data was subjected to Students't' test.

RESULTS:

3-Hydroxy-3-methylglutaryl Co A reductase Assay: In the present study, liver microsomes were treated with MO polyophenols at 200, 300 and 400µg along with a

control and their effect on the activity of 3-hydroxy-3-methylglutaryl CoA reductase was measured based on the coenzyme A released during the reduction of 3-hydroxy-3-methylglutaryl CoA to mevalonate. Results indicate that the MO polyphenols were effective in inhibiting the HMG CoA reductase in the liver microsomes.

The Coenzyme A released during the assay was significantly ($p<0.05$) higher (18.87±0.12 nMoles/mg protein) than the microsomes treated with MO polyphenols at different concentrations (**Figure 1 and 2**) and polyphenols at 400 µg were more potent in inhibiting the enzyme activity (10.26±0.33 nMoles/mg protein).

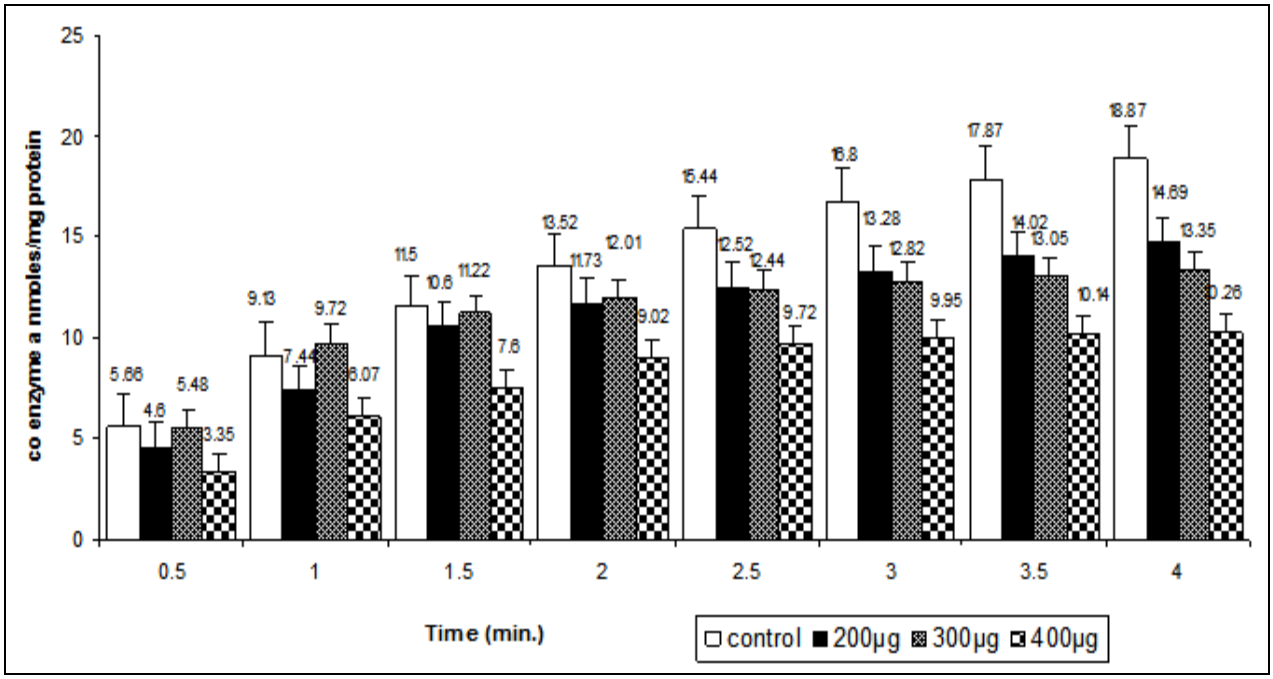

FIG. 1: THE EFFECT OF MO POLYPHENOLS ON INHIBITING THE ACTIVITY OF HMG CoA REDUCTASE AND RELEASE OF Co ENZYME A BY THE REDUCTION OF HMG CoA TO MEVALONATE

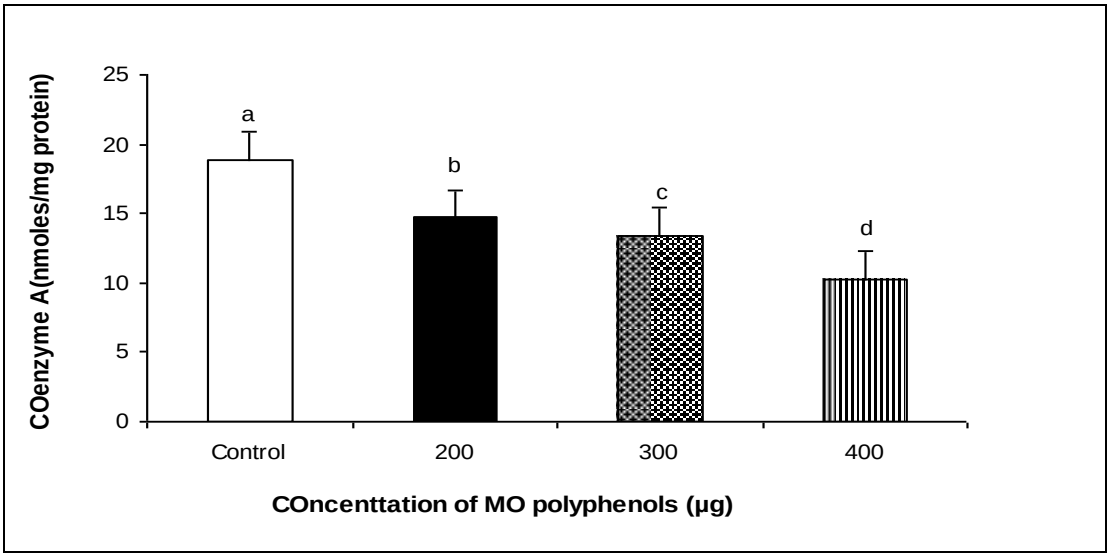

FIG. 2: TOTAL Co ENZYME A (n moles/mg PROTEIN) RELEASED DURING THE REDUCTION OF HMG CoA TO MEVALONATE

Figure 1 shows the time course of activity of HMG CoA reductase for a period of 4 minutes, where we can observe that the rate of release of Coenzyme A was constantly high from the zero to 4th minute (5.66 to 18.87nMoles) than the polyphenol treated tubes. At 200 and 300 µg concentration differences were observed in the rate of release of Coenzyme A in the first 2 min, lesser amount was released with 200µg concentration (11.7nMoles) than 300µg (12.01 nMoles). However, after the third minute more Coenzyme A released at 200 µg (13.28 nMoles) was more than the 300 µg (12.82 nMoles).

With 400µg polyphenol extract, the rate of release of Coenzyme A was constantly less (3.35-10.26nMoles) from the zero to 4th minute, compared to control and other variations.

Fig. 2 shows the total HMG Co A reductase activity as nMoles of reduced Coenzyme A released at the end of 4th minute. The polyphenols at all the concentrations were potent in inhibiting the activity of HMG Co A reductase enzyme than the control group and also it can be observed that there is a significant difference ($p<0.05$) in between the dosages (200µg- 14.69±0.07, 300µg- 13.37±0.16, 400 µg -10.26±0.33 nMoles) of the polyphenols in regulating the HMG CoA reductase activity, which shows even at lower dosages the poyphenols have actively inhibited the enzyme activity.

DISCUSSION: The cholesterol homeostasis is maintained by the control of the two processes, viz. cholesterol biosynthesis in which HMG CoA reductase catalyses the rate limiting process and cholesterol absorption of both dietary cholesterol and cholesterol cleared from the liver through biliary secretion. The hepatic activity of HMG CoA reductase is controlled by the nutritional and hormonal state of animals [19].

It is known that feedback suppression of cholesterol is mediated through changes in the activity of HMG CoA reductase. Changes in the reductase activity are closely related to changes in the overall rate of cholesterol synthesis, suggesting that the inhibition of HMG-CoA reductase would be an effective means of lowering plasma cholesterol in humans [6]. From past many years various HMG CoA reductase-inhibitors have been identified from many herbs, medicinal plants, microorganisms etc.

Meanwhile, several chemically synthesized compounds, fluvastatin, cerivastatin, strovastatin, rosuvastatin, and HR780, have also been developed as effective cholesterol lowering agents through the inhibition of HMG CoA reductase. Similarly, some chemically modified HMG CoA reductase-inhibitors, including simvastatin and pravastatin, have been developed from natural products. However, most commercial HMG CoA reductase-inhibitors have been found to possess adverse effects, including the induction of cutaneous vasodilation, rashes, gastrointestinal discomfort, hyperuricemia, hyperglycemia and hepatic dysfunction [20]. There are many reports, on the hypocholesterolemic potency of herbal drugs [3]. Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of CVD [7].

In our lab, a study was conducted by supplementing *Moringa* leaf powder as a source of vitamin E resulted in a significant decrease in plasma cholesterol compared to the 2 control diets where Palm oil and pea nut oil was fed to the rats. Palm oil is known to exhibit hypocholesterolemic effect [21], Supplementing both PO and PN diets with higher levels of vitamin E from *Moringa* proved to be beneficial in lowering plasma cholesterol [11]. *Moringa oleifera* leaf and fruit have been reported to exhibit hypocholesterolemic effect in rats and rabbits [9, 11, 22]. Both studies observed a reduction in serum cholesterol levels in the animals. However, the mechanism involved in the cholesterol lowering by *Moringa oleifera* leaf and fruit has not been reported.

Hence, an attempt was made to determine the role of MO leaves on the cholesterol biosynthesis by *ex vivo* treatment of the liver microsomes with MO polyphenols at different concentrations (200-400 µg). In rat liver microsomes, a cell free system, MO polyphenols inhibited HMG CoA reductase activity. This is the first report on the *ex vivo* HMG CoA reductase inhibition by *Moringa oleifera* polyphenols. Phenolic compounds are now widely accepted as antioxidants that have a significant potential to protect against many degenerative diseases linked to free radical related tissue damage [23].

The inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which is the enzyme responsible for conversion of HMG CoA to mevalonate and a rate limiting step in cholesterol biosynthesis, can achieve relatively large reductions in plasma cholesterol levels and represent an established class of drugs for the treatment of hypercholesterolemia. Several large prospective clinical trials have recently shown that treatment of patients with and without coronary heart disease (CHD) with HMG CoA reductase inhibitors as well as other lipid-lowering drugs such as fibrates to lower plasma cholesterol levels, leads to a reduction of cardiovascular morbidity and mortality [24, 25, 26].

The use of substances inhibiting HMG CoA reductase (statins) is very effective in blood cholesterol reduction. In a study, focused on the effect of the polyphenolic compounds from virgin olive oil on cholesterol metabolism, have demonstrated a significant decrease in the activity of HMG CoA reductase in the liver microsomes of rats. The inhibition of HMG CoA reductase by polyphenolic compounds represent a beneficial effect through olive oil ingestion and can play an important role in the prevention of cardiovascular diseases. [27].

Similarly, Hirangi *et al*., [3] have analyzed the *in vitro* inhibition of the HMG CoA reductase by several herbal drugs known to be potent antihyperlipidemic agents. Among different medicinal plants *Andrographis paniculata*, *Anthocephalus indicus* and *Terminalia arjuna* at 0.05 and 0.150 mg possessed HMG CoA reductase inhibitory activity.

With increased interest in dietary phenolic compounds in cereals, herbs, fruits, roots and vegetables as protective agents against coronary heart disease and cancer [3, 6, 28], results obtained in the present investigation show that the anti HMG CoA reductase activity of MO polyphenols encourages designing an *in vivo* study on animal models to confirm their effects. It would be interesting to purify and characterize the active constituents of these extracts, to establish the composition and mechanism of action and to confirm their pharmacological potentials.

CONCLUSION: The results of the present study show the role of polyphenols in modulating the cholesterol metabolism by inhibiting the activity of HMG CoA reductase. This finding lends support to the reported studies on the hypocholesterolemic role of *Moringa oleifera* leaves.

Studies on the isolation and characterization of pure MO polyphenols can result in developing a neutraceutical or therapeutic agent and further they can be used in functional food formulations in the management of disorders of cholesterol for controlling the dyslipidemia.

ACKNOWLEDGEMENT: The author thanks Department of Science and Technology (DST) New Delhi, for financial assistance.

REFERENCES:

1. American Heart Association, AHA dietary guidelines. Revision: *A* statement *for* healthcare professionals from the nutrition committee of the American Heart Association, Circulation 2000; 102:2296-2311.
2. Satyanarayan U: Metabolism of lipids, Textbook of Biochemistry, 3rd ed. Books and Allied Pvt. Ltd, 2006; 309-312.
3. Patel HD, Shah GB, Trivedi V: 1Investigation of HMG Co A Reductase Inhibitory Activity of Antihyperlipidemic Herbal Drugs In Vitro Study. Asian Journal of Experimental Biological Sciences 2011; 1: 63-68.
4. Zuraini A, Aziah MR, Arifah AK, Sulaiman M. Somchit MN: Aqueous Extract of *Andrographis paniculata* improve Lipid profile of rats Fed with High cholesterol diet. International journal of Pharmacology 2006; 2(1): 45-49.
5. Miliauskas G, Venskutonis PR, Van Beek TA: Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85:231-237.
6. Visavadiya NP. Narasimhacharya AVRL: Asparagus Root Regulates Cholesterol Metabolism and Improves Antioxidant Status in Hypercholesteremic Rats. Advance Access Publication 2009; 6(2):219–226. (http://creativecommons.org/licenses/by-nc/2.0/uk/)
7. Shahidi, F. Janitha P.K., Wanasundara P.D: Phenolic antioxidants. Critical Review in Food Science and Nutrition 1992; 202:307.
8. Morton LW, Caccetta RA, Puddey IB Croft KD: Chemistry and Biological Effects of Dietary Phenolic Compounds: Relevance to Cardiovascular Disease. Clinical Experimental Pharmacology and Physiology 2000; 27(3): 152-159
9. Ghasi S, Nwobodo E, Ofili JO: Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high – fat diet fed Wister rats. Journal of Ethnopharmacology 2000; 69: 21- 25.
10. Bour S, Visentin V, Prévot D, Daviaud D, Saulnier-Blache JS, Guigne C, Valet P, *Carpéné* C: Effects of oral administration of benzylamine on glucose tolerance and lipid metabolism in rats. Journal of Physiology and Biochemistry 2005; 61(2): 371-379.
11. Oinum, N, Urooj, A, Philips, P.P., Niranjan NP: Effect of dirtary lipids and Drum stick leaves (Moringa oleifera) on liid profile and antioxidant parametrs in rats. Food Nutrition Sciences, (Accepted) [online] Feb 2012, http;//www.sciRP.org/ journal/fns.
12. Arabshahi-Delouee, S, Devi V, Urooj A: Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chemistry 2007; 100: 1100-1105.
13. Urooj A, Reddy PV: *Moringa oleifera*: antioxidant properties and stability of various solvent extracts. International Journal of Pharmaceutical sciences and Biotechnology [online] 2010;.1(1):1-6.Available at http://www.ijpsb.com.
14. Reddy PV, Urooj A, Kumar A: Evaluation of antioxidants activity of some plant extracts and their application in biscuits , Food Chemistry 2005; 90: 317-321.
15. Arabshahi-Delouee S, Urooj A. MO Application of Phenolic Extracts from Selected Plants in Fruit Juice. International Journal of Food Properties 2007; 10 (3): 479-488.
16. Rich A, Rich C, University of Louisville, Louisville, KY: Extraction of Chlorophyll and Carotenes from Spinach. and Analysis by Thin Layer Chromatography. Physiological Chemistry-Extraction of Chlorophyll from Spinach. Toni Bell, Bloomsburg University. 1-6.
17. Shapiro DJ, Rodwell VW: Regulation of hepatic 3-hydroxy-3-methylglutaryl Coenzyme A reductase and cholesterol

synthesis. Journal of Biological Chemistry 1971; 246: 3210-3216.
18. Frankel H, Hulcher and Wayne H: OlesonSimplified spectrophotometric assay for microsomal 3-hydroxy-3-methylglutaryl Coenzyme A reductase by measurement of coenzyme. A. Journal of Lipid Research 1973; 14: 625-631.
19. Ide T, Sugano OHM: Regulation of dietary fats of 3 hydroxy 3 methylglutaryl Co enzyme A reductase in rat liver. Journal of Nutrition 1978; 108:601-612.
20. Yu H-E, Lee D-H, Seo G-S, Cho S-M, Lee, J-S: Characterization of a Novel β-Hydroxy-β- methyl Glutaryl Coenzyme A Reductase-Inhibitor from the Mushroom, Pholiota adipose. Biotech Biopro Engineering 2007; 12:618-624.
21. Khor HT, Daniel TS, and Tan: Studies on the lipidemic property of dietary palm oil: Comparison of the responses of serum, liver and heart lipids to dietary palm oil, palm oil triglycerides, coconut oil and olive oil. Nutrition Research, 1992; 12:105.
22. Mehta K, Balaraman R, Amin AH, Bafna PA, Gulati OD: Effect of fruits of Moringa oleifera on the lipid profile of narmal and hypercholesterolmic rabbits. Journal of Ethnopharmacology 2003; 86:191-195.
23. Dreosti,IE: Antioxidant polyphenols in tea, cocoa and wine. Nutrition 2000; 16:671-692.
24. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). Lancet 1994; 344:1383–1389.
25. Shepherd J, Cobbe SM, Ford I, et al. West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with provastatin in men with hypercholesterolemia. New England Journal of Medicine 1995; 333:1301–1307.
26. Stael, B. Dallongeville J; Auwerx, J; Schoonjans, K; Leitersdorf E; Fruchart J-C: Mechanism of Action of Fibrates on Lipid and Lipoprotein Metabolism. Cardiovascular Drugs 1998; 98:2088-2093.
27. Benkhalti F, Prost J, Paz E, Perez-Jimenez F, El Modafar C. El Boustani E: Effects of feeding virgin olive oil or their polyphenols on lipid of rat liver. Nutrition Research 2002; 22:1067–1075.
28. Kamal-Eldin A, Frank J, Razdan A, Tengblad S, Basu S, Vessby B: Effects of Dietary Phenolic Compounds on Tocopherol, Cholesterol, and Fatty Acids in Rats. Lipids 2000; 35:427-435.

How to cite this article:
Reddy VP, Ahmed F and Urooj A: Inhibition of 3-hydroxy-3-methylglutaryl coenzyme a (HMG Co-A) reductase in liver microsomes by *Moringa oleifera* l. Polyphenols. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 2510-2516.