Effect of pectin derived from *Averrhoa carambola L.* (Carambola) in the lipid profile of Sprague-Dawley rats

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ABSTRACT

Carambola (*Averrhoa carambola L*.) fruit is commonly known as star fruit because of its unique star- like appearance and rich golden color. This fruit is distributed in tropical and subtropical regions. In some Asian countries, the mature fruit is eaten fresh and relished in pickle preparations. It is rich in antioxidants and vitamin C and low in sugar, sodium and acid. It is also a potent source of both primary and secondary polyphenolic antioxidants. It also contains pectin that has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food. This study, therefore, determined the effect of pectin derived from carambola fruit in the lipid profile of Sprague-Dawley rats. Four-week administration of 150mg of pectin to the laboratory animals lowered their serum total cholesterol, very low density lipoprotein (VLDL), low density lipoprotein (LDL) levels of the laboratory animals while high density lipoprotein (HDL) increased. A p-value of <0.05 shows no significant difference on the effect of 150mg of pectin against 10mg of Atorvastatin calcium. Further studies on the effects of pectin, especially on serum HDL level, is recommended.

Keywords: star fruit, pectin, lipoproteins, rats, carambola

INTRODUCTION

Averrhoa carambola L., also known as star fruit, belongs to family Oxalidaceae. It is a tropical common evergreen woody fruit plant that is native to South East Asia and presently being grown as a garden tree for its edible fruits (Flora, 2004; 2008) in all tropical and sub-tropical countries. It has many commercial exploitable and beneficial attributes (Tidbury; Roy, 2007).

The star fruit plant is a small shrub-like ornamental tree with edible fruit that is rich in vitamins, phosphorus and oxalic acid (Moreira, 1985 as cited by Vicentini, 2001). As reported by Shui and Leong (2006), star fruit residues which only contributed to 15% of its total weight, account for up to 70% of total antioxidant activity and total polyphenolic contents. Fruit juices contain colloids that are mainly polysaccharides (pectin, cellulose, hemicelluloses, lignin, and starch), protein, tannin and metals (Vaillant et al., 2001; Abdullah, 2006). It is

known for its anti-diabetic and anti-hypertensive effect. It is also has an appetite stimulator, anti-diarrhea, anti-scurvy and antipyretic attributes (Moreira, 1985; Vicentini, 2001). Additionally, it has been used topically on poisonous bites and stings (Lamoni, 1997; Vicentini, 2001).

Averrhoa carambola L. is also a source of phenolic compounds that posses a wide span of physiological functions including antioxidant, anti-carcinogenic, anti- inflammatory, anti-allergenic, anti-microbial, anti- thrombotic, cardio-protective and vasodilatory effects to a greater or lesser extent (Pathriana & Shahidi, 2005; Balasundram et al. 2006; Cai et al. 2006; Rodrigues et al., 2008; Ho, 2009).

Pectin is a family of complex variable polysaccharide extracted from the primary cell wall of higher plants. Chemically, pectin consists of linear polymers of D-α (1-4) anhydrogalacturonic acid (Schemin, 2005). Pectins are soluble in pure water. Monovalent cation (alkali metal) salts of pectinic and pectic acids are usually soluble in water; di- and trivalent cations salts are weakly soluble or insoluble (Rolin, 1993; Sriamornsak, 2002). Pectins are specific plant polysaccharides (Selivanov, 2008). It is most concentrated in citrus fruits like oranges, lemons, grapefruits and apples. Pectin obtained from citrus peels is referred to as citrus pectin (Sharma, 2006).

Pectin is a high value functional food ingredient widely used as gelling agent and stabilizer (Pinhiero et al., 2008) The main raw materials used to produce commercial pectin are apple pomace and citrus peels (Kliemann, 2009). In human diet, pectin occurs as “soluble fiber” in fruit and vegetables, jams and jellies and more recently in low calorie foods as a fat replacer (Thakur et al*.,* 1997; Marounek, 2004). Pectin is used not only in food processing in jam, jellies, marmalades, ketchups, sauces, juices, concentrate syrups and yoghurt, but also in pharmaceutical preparation such as medical formulations for stabilizing the suspensions. It also has an important role in cosmetic industry (Virk and Sogi, 2004).

Pectin is synthesized in Golgi vesicles and its complexity dictates that a large numbers of enzymes must be involved in the process. Enzymes that degrade parts of the pectin can also be used to release pectin (Scheller, 2007).

According to Sharma 2006, fruits and vegetables are especially rich in pectin that have dietary fiber contents in the range of 1-2% which shows that pectin can be a source of dietary fiber. In addition to this, pectin has prebiotic effects. Some fermentation of pectin takes place in the large intestines via the action of bacteria. Modified citrus pectin, when administered orally, to rats was found to inhibit spontaneous prostate carcinoma metastasis which shows anti-carcinogenic effects. Another important action of pectin is its hypocholesterolemic activity. The hypocholesterolemic effect of dietary pectin has been reported in studies with humans, mice, guniea pigs, hamsters, rabbits, chickens, and pigs. (Terpstra, 2002). Pectin is frequently regarded as a very potential carbohydrate to decrease serum

cholesterol. (Abdelbaky et al., 2009).There are no published literatures however on the effects of pectin derived from carambola.

Hypercholesterolemia has been proven to be one of the most important risk factors for atherosclerotic vascular diseases (Adaromoye, 2005). It is a primary risk factor in the development of cardiovascular disease. It is well established that "Western diets," known for their high fat, high cholesterol, excess energy, and low fiber contents, increase serum cholesterol levels (Bush, 1988; Yang, 2002).

Liver plays a central role in lipoprotein metabolism (Xu, 2007). It synthesizes vital substances called cholesterol which is the building block of essential organic molecules such as steroid hormones, cell membranes, and bile acids that are used by the body. However, its excessive accumulation leads to coronary heart diseases (Butt, 2007).

Cholesterol is found in foods of animal sources, for example, egg yolks, meat and cheese. Pure cholesterol is essential for cell survival, whereas even small amounts of oxidized cholesterol produce significant toxicity (Yeh et al., 2007).

Lipoproteins are substances of high molecular weight which ranges up to 60000-10^{11}. Its density is less than that of the serum proteins such as albumin and several globulins. Difference in density among like lipoproteins allows for segregation of the lipoproteins into three general density ranges. Plasma lipoproteins including high-low intermediate and very low density lipoproteins (HDL, LDL, IDL, and VLDL) are macro molecular complexes of lipids and proteins (termed apolipoproteins) that mediate lipid transport and metabolism and are central in the development of coronary artery disease. HDL is anti-atherogenic, LDL is pro-atherogenic and VLDL is not only direct metabolic precursor of LDL but also an independent risk factor for atherosclerosis (Guha, 2007). Major risk factors for atherosclerosis include high plasma LDL concentrations and LDL modifications such as its retention, oxidation and aggregation (Williams and Tabas, 1995; Hossin, 2009).

The present study determined the effect of pectin derived from carambola fruit to the lipid profile using egg yolk diet for 2 weeks in Sprague-Dawley rats and compared to the effect of pectin against the standard drug, atorvastatin calcium.

MATERIALS AND METHODS

The carambola fruit used in the experiment was obtained from Rosario, Batangas. Ripe carambola fruits with yellowish color were chosen and the spoiled fruits were discarded. Vouchers of the plant specimen were submitted to the Thomas Aquinas Research Complex Herbarium of the University of Santo Tomas with authentication no. USTH-5547.

Pectin extraction

The extraction procedure was based on that of Kratchanova et al. (2004) as cited by Kliemann et al. (2009). A dry mass (5 g) was subjected to extraction by adding 250 mL of water. The pH was adjusted to 1.2–2.6 with 0.5 M HCl. The mixture was then heated to 90 °C and the extraction was carried out with continuous stirring for 90 min. The hot acid extract was filtered through an ordinary screen with 1-mm mesh size equipped with two-layer cheesecloth, and the filtrate was cooled down to 4 °C. The filtrate was coagulated using an equal volume of 96% ethanol and left for 1 hour. The coagulated pectin was separated by filtration, washed once with 70% acidic ethanol (0.5% HCl), then with 70% ethanol to a neutral pH and finally with 96% ethanol. The resulting material was dried overnight at 55°C in a gravity convection oven.

Animals and Diets

Twelve adult Sprague-Dawley rats with an average weight of 200 to 300g were obtained from BioPhilippines Manila. The rats were housed in suspended steel wire cages and the environment was controlled in terms of light (12:12h light/dark cycle starting at 6:00 AM), humidity and room temperature (20-25°C) (Aubin et. al., 2008). All rats had *ad libitum* access to food and water (Abdullah, 2009).

Hypercholesterolemia was induced in rats by feeding the rats with 100 mg of egg yolk per day for 2 weeks before the beginning of the experiment and during the experiment (Metwally et al., 2009).

Three groups of rats were used in the study. The animals of group I (5 members) served as the positive control and received egg yolk and commercially available drug, atorvastatin calcium, as a positive control. Group II (2 members) was the negative control and received egg yolk and waterduring the experiment. Group III (5 members) received egg yolk and pectin extracted from carambola fruit. All procedures sought the ethical approval of the Bureau of Animal Industry for all laboratory animals that was used in the study.

Sample collection

Blood samples were collected before feeding the rats on the experimental diet, every two weeks during the experiment and at the end of the experimental period. After overnight fasting, blood samples were collected from each rat from the tail into microtainer tubes (Park et al., 2007). The blood was allowed to clot at room temperature for one hour, and then the serum was separated by centrifugation at 3500 rpm for 5 minutes, clear serum was stored at 20°C until analysis (Abdel Ati et al., 2009). Commercial kits from ELITECH, Zone Industrielle, France were used for the determination of serum levels of total cholesterol, High Density Lipoproteins (HDL) and triglycerides using a semi-automated spectrophotometer (StatFax, Awareness Technology, USA).

Low density lipoprotein (LDL) and Very-Low Density Lipoprotein (VLDL) were computed using Fridewald's equation (Mcpherson, 2001; Henry, 1999).

The Analytical Methods of Serum

Determination of Total Cholesterol: The cholesterol level was measured by enzymatic method (ELITECH, Zone Industrielle, France).

Determination of High Density Lipoprotein (HDL) cholesterol: Chylomicrons, Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) of serum were precipitated by phosphotungstic acid and magnesium ions. After centrifugation, high density lipoproteins (HDL) were in the supernatant. Cholesterol included in this phase, was measured by an enzymatic method (ELITECH, Zone Industrielle, France).

Determination of Triglycerides: The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator used was quinoneimine which was formed from hydrogen peroxide, 4-aminophenazone and 4- chlorophenol under the catalytic influence of peroxidase (ELITECH, Zone Industrielle, France).

Determination of Very Low Density Lipoprotein (VLDL) cholesterol: The concentration of VLDL was computed according to the Fridewald's equation:

VLDL = triglyceride/5

Determination of Low Density Lipoprotein (LDL) cholesterol: According to Fridewald *et al*., 1972, lowdensity lipoprotein cholesterol can be calculated asfollows:

LDL = Total cholesterol - (HDL) - (VLDL)

Statistical analysis

Results were expressed as means ± SE and statistical analyses were performed using ONEWAY ANOVA by SPSS statistical software package (SPSS for Windows, 10.0). *p*values less than 0.05 were considered significant (Manjunatha and Srinivasan, 2007).

RESULTS AND DISCUSSIONS

Pectin from *Averrhoa carambola* has been found to have effects on selected serum lipid analytes of the laboratory animals. The baseline of the serum total cholesterol of the test and positive group was 376.92 and 269.28 mg/dl respectively. After the induction of

hypercholesterolemia, the serum level of the test group rose to 5478.40 and 4648.40 mg/dl for the positive group. After four weeks, two tests were done and the serum total cholesterol levels decreased to 790.64 and 612.92 mg/dl for the test group and the positive group decreased to 774.72 and 523.72 mg/dl. The negative group with the baseline of 413.30 rose to 1030.50 mg/dl after the induction of hypercholesterolemia and continuously increased to 1579 mg/dl and 2572 mg/dl after 4 weeks of testing.

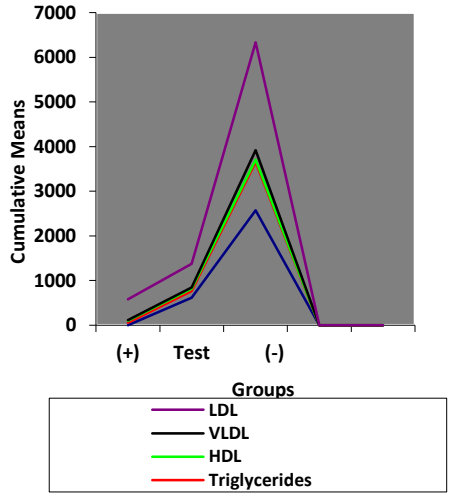

Figure 1. Effect of pectin and Atorvastatin calcium on Total cholesterol, triglycerides, HDL, VLDL and LDL.

With triglycerides, baseline levels of the three groups (test, positive, negative) was 167.86, 176.26, 301.50 mg/dl respectively. The serum TAG levels rose to 748.7, 425.62, 796.40 mg/dl after the induction of hypercholesterolemia. After 4 weeks of testing, the test group decreased to 369.22 and 151.9 mg/dl and the positive group decreased to 200.04 and 57.82 mg/dl. On the other hand, the negative group increased to 907.50 and 1083.85 mg/dl.

A baseline of 33.57, 35.25, 60.30 mg/dl was obtained from the serum's very low density lipoprotein (VLDL). After high-fat diet, the serum levels rose to 135.34, 85.12, 159.28 mg/dl.After 4 weeks of testing, the levels for the test group decreased to 73.84 and 30.38 mg/dl and the positive group decreased to 40.01 and 11.56 mg/dl while the negative group increased to 181.50 and 216.77 mg/dl.

Moreover,the baseline for low density lipoprotein of the three groups (test, positive, negative) were as follows: 342.43, 233.17,

351.10 mg/dl. After the induction of hypercholesterolemia, the levels rose to 5369.90, 3369.70 and 868.92 mg/dl. Two testings were also done after 4 weeks and yielded the following results: 709.86 and 533.88 mg/dl for the test group; 729.47 and 469.34 mg/dl for the positive group; 1393.15 and 2412.49 mg/dl for the negative group.

Unlike the four previous parameters, the levels of high density lipoprotein (HDL) of the three groups (test, positive, negative) increased throughout the testing. The baseline were as follows: 0.92, 0.86, 1.90 mg/dl. After 4 weeks of testing, the levels rose to 5.14 and 48.66 mg/dl for the test group; 5.24 and 42.82 mg/dl for the positive group. Lastly, for the negative group, the levels rose to 4.35 and 49.45 mg/dl.

There was a significant difference on the serum cholesterol, triglycerides, VLDL and LDL levels of treated rats in the positive, negative and test groups on the two-week feeding as indicated by the f-value of 24.36 at 0.05 level of significance. Based on the multiple comparison of the means, the serum cholesterol, triglycerides, VLDL and LDL levels of rats under the test groups differ significantly with rats under the negative control groups but the serum cholesterol, triglycerides, VLDL and LDL levels of test groups do not differ with the positive controls. Based on the results gathered, the test and positive groups show similar effects on the lipid profile of the serum tested. Both Atorvastatin calcium and pectin lowers the serum total cholesterol, TAG, VLDL, LDL. Furthermore, statistical analysis of results revealed that the effect of 150 mg of pectin on serum levels of cholesterol, triglycerides, VLDL and LDL is similar to the therapeutic effect of 10 mg of Atorvastatin calcium.

Using 150 mg of pectin, the serum HDL level of the laboratory animals showed an increasing trend. However, since the p-values were all higher than 0.05 level of significance, the computed f-ratios were found to be not significant. There was no significant difference on the HDL level of rats of positive control, test and negative controls in terms of the baseline data, during the first, second and third feeding. Therefore, both Atorvastatin calcium and pectin do not affect the high density lipoprotein during the 4-week testing period.

The lowering effect of carambola on the selected serum lipid analytes may be explained by its pectin component. Its fruit juices contain colloids that are mainly polysaccharides which includes pectin (Vaillant *et al.*, 2001; Abdullah, 2006). Pectin is frequently regarded as a very potential carbohydrate in decreasing serum cholesterol levels (Abdelbaky et al., 2009). Its effects on other components of the lipid profile however are limited. This study affirms its lowering effect on cholesterol and establishes the effect of pectin on triglycerides, VLDL, LDL and HDL. Reported studies on the effects of pectin utilized artificial

purified pectin. Results of this study establish the similar effect of pectin on lipid analytes derived from carambola.

CONCLUSION

Pectin, derived from carambola fruit, has a lipid lowering effect on cholesterol, triglycerides, VLDL and LDL on the serum of Sprague-Dawley rats. On the other hand, HDL is not affected by all experimental groups. A 150mg of pectin has shown similar effects with that of 10mg of atorvastatin calcium on serum levels of cholesterol, triglycerides, LDL and VLDL thus suggesting the comparability of their therapeutic effects.

RECOMMENDATIONS

Future researchers are recommended to conduct further studies on the effects of pectin using a more purified form. In testing for HDL, it is recommended to use a larger population of laboratory animals.

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REFERENCES

1. Abdel Ati, Khadiga A., Mustafa, A.E. & Mohamed, H.E. (2009). The Effect of Dietary *Nigella sativa* Seeds on the Blood Cholesterol and Lipoprotein Levels of Rabbits. Journal of Animal and Plant Sciences. Vol. 3, Issue 3: 227-230.
2. Abdelbaky, M.S., Elmehiry, H.F. & Ali, N.K.M. (2009) Effect of some citrus peels on hypercholesterolemic rats
3. Abdullah, Liew A.G., Sulaiman, N.M., Aroua, M.K. & Megat Mohd Noor, M.J. (2006). Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme.
4. Abdullah, M.Z., Fathinul-Syahir, A.S. & Mohd-Azemi, B.M.N. (2005). Automated Inspection System for Colour and Shape Grading of Star Fruit (Averrhoa carambola L.) using machine vision sensor. Transactions of the Institute of Measurement and Control 27, 2 pp. 65-87.
5. Abdullah Y.A. & Al-Rewashdeh (2009). Lipid Profile of Rats Fed Cholesterol, Barely and Wheat. Pakistan Journal of Nutrition 8 (11): 1722-1733.

6. Adaramoye, O. A., Nwaneri, V.O., Anyanwu, K.C., Farombi, E.O. & Emerole, G.O. (2005). Possible Anti-Atherogenic Effect of Kolaviron (*A Garcina Kola* Seed Extract*)* in Hypercholesterolaemic Rats. Clinical and Experimental Pharmacology and Physiology*,* 32, 40–46.
7. Aubin, Marie-Claude, Lajoie, C., Clement, R., Calderone, A. & Perrault, L.P. (2008). Female Rats Fed a High-Fat Diet Were Associated with Vascular Dysfunction and Cardiac Fibrosis in the Absence of Overt Obesity and Hyperlipidemia: Therapeutic Potential of Resveratrol.
8. Balasundram, N., Sundram, K. & Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant Activity, Occurrence, and Potential Uses. Food Chemistry 99: 191-203.
9. Bush, T. L., L. P. Fried, and E. Barett-Connor. 1988. Cholesterol, Lipoproteins, and Coronary Heart Disease in Women. Clin. Chem. 34: B60-B70.
10. Butt, M.S*.,* Ahmad, A. & Sharif, M.K., (2007). Influence of Pectin and Guar Gum Composite Flour on Plasma Biochemical Profile of Streptozotocin-induced Diabetic Male Albino Rats. International Journal of Food Properties, 10:345-361.
11. Fatma, L. & Hossin, A. (2009).Effect of Pomegranate (*Punica granatum*) Peels and It's Extract on Obese Hypercholesterolemic Rats. Pakistan Journal of Nutrition 8 (8): 1251-1257.
12. Fridewald, W.T., R.I. Leve and D.S. Fredrickson, (1972). Estimation of the Concentration of Low Density Lipoprotein. Clin. Chem., 18: 499-502.
13. Guha, Madhumita, England, C., Herscovitz, H. & Gursky, O. (2007). Thermal Transitions in Human Very Low Density Lipoprotein: Fusion, Rupture and Dissociation of HDL-Like Particles.Biochemistry. 46(20): 6043–6049. Doi: 10.1021/bi7001532.
14. Henry's Clinical Diagnosis and Management by Laboratory Methods. 21st Edition 2007. McPherson RA, Pincus MR. (Eds). Saunders, Philadelphia.
15. Kliemann, E., de Simas, K.N., Amante, E.R., Prudencio, E.S., Teofilo, R.F., Ferreira, M.M.C. & Amboni, R.D.M.C., (2009). Optimisation of Pectin Acid Extraction from Passion Fruit Peel (Passiflora edulis flavicarpa) Using Response Surface Methodology. International Journal of Food Science and Technology, 44, 476–438.
16. Kratchanova, M., Pavlova, E. & Panchev, I. (2004). The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. Carbohydrate Polymers, 56, 181–185.

17. Lamoni, R. A cura pelos remédios caseiros. Rio de Janeiro: Ediouro, 1997.
18. Manjunatha H. and Srinivasan K., (2006). Hypolipidemic and Antioxidant Effects of Curcumin and Capsaicin in High-fat-fed Rats.
19. Marounek M., Volek, Z., Synytsya, A. & Copikova, J. (2004). Effect of Pectin and Amidated Pectin on Cholesterol Homeostasis and Caecal Metabolism in Rats Fed a High-Cholesterol Diet.Research Institute of Animal Production, Prague.
20. Metwally, M.A.A., El-Gallal, A.M. & El-Sawaisi, S.M. (2009). Effects of Silymarin on Lipid Metabolism in Rats. World Applied Sciences Journal 6 (12): 1634-1637.
21. Moreira, F. As Plantas que curam. Brasil: Hemus Ed., 1985. Flora: A Gardener's Encyclopedia:Volume I. Garden Cheers Publishing (2004).
22. Pathirana, C. L. & Shahidi, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry 93: 47-56.
23. Park, Yoon Shin, Yoon, Y. & Ahn, H.S. (2007). Platycodon Grandiflorum Extract Represses Up-regulated Adipocyte Fatty acid binding Protein triggered by a High Fat Feeding in Obese Rats. World Journal of Gastroenterology: 13(25):3493-3499.
24. Pinhiero, E.R., Silva, I.M.D.A., Gonzaga, L.V., Amante, E.R., Teofilo, R.F., Ferreira, M.M.C. & Amboni, R.D.M.C., Optimization of Extraction of High-ester Pectin from Passion Fruit Peel (Passiflora edulis flavicarpa) with Citric Acid by Using Response Surface Methodology. Bioresource Technology 99: 5561–5566.
25. Rodrigues, S., Pinto, G.A.S. & Fernandes, F.A.N. 2008. Optimization of Ultrasound Extraction of Phenolic Compounds from Coconut (Cocos nucifera) Shell Powder by Response Surface Methodology. Ultrasound Sonochemistry 15: 95-100.
26. Rolin, C. (1993). Pectin In Industrial gums, eds. R.L. Whistler and J.N. BeMiller. 3rd edn. (New York: Academic Press)
27. Roy, P. K., Mamun, A.N.K. & Ahmed, G. (2007). Rapid Multiplication of Averrhoa Carambola through In Vitro Culture. J. bio-sci. 15: 175-179.
28. Scheller, Henrik Vibe, Jensen, J.K., Sorensen, S.O., Harholt, J. & Geshi, N. (2007). Biosynthesis of Pectin. Physiologia Plantarum 129: 283–295.
29. Schemin, Maria Helene Canteri, Fertonani, H.C.R., Wsazczynskyj, N. & Wosiacki, G. (2005). Extraction of Pectin from Apple Pomace. Brazillian Archives of Biology and Technology,Vol.48, n. 2: pp. 259-266.

30. Selivanov, N. Yu., Sorokina, I.V., Selivanova, O.G., Sokolov, O.I. & Ignatov, V.V. (2008). Investigation of Enzymatic Degradation of Pectin Polysaccharidesunder Limiting Conditions. ISSN 0006_2979, Biochemistry (Moscow), Vol. 73, No. 1, pp. 80_86. © Pleiades Publishing, Ltd., 2008.
31. Sharma, B.R., Naresh, L., Dhuldhoya, N. C., Merchant, S.U. & Merchant, U.C. (2006).An Overview on Pectins. Times Food Processing Journal, June-July Issue, Page no. 44-51.
32. Sriamornsak, P., (2002). Chemistry of Pectin and Its Pharmaceutical Uses: A Review.
33. Terpstra, A. H. M., Lapre, J.A., de Vries, H.T. & Beynen, A.C. (2002). The Hypocholesterolemic Effect of Lemon Peels, Lemon Pectin, and the Waste Stream Material of Lemon Peels in Hybrid F1B Hamsters. Eur J Nutr 41: 19–26.
34. Thakur B. R., Slugh R. K., Handa A. K. (1997). Chemistry and uses ofpectin- A Review. Crit Rev Food Sci 37: 47-73.
35. Thomas S., Patil, D.A., Patil, A.G. & Naresh, C. (2008). Pharmacognostic Evaluation and Physicochemical Analysis of *Averrhoa carambola L*. Fruit.of Herbal Medicine and Toxicology 2 (2) 51-54 ISSN: 0973-4643.
36. Tidbury G.E. (1976) Averrhoa spp.- Carambola and Bilimbi. In: The Propagation of Tropical Fruit Trees. Garner R J and Chaudhary S A (eds). FAO, CAB, London. pp. 291-310.
37. Vaillant, Millan, Dornier, Decloux, & Reynes. (2001). Strategy for Economical Optimization of the Clarification of Pulpy Fruit Juices Using Cross Flow Microfiltration. Journal of Food Engineering, 48,83–90.
38. Vicentini, Veronica Elisa P., Camparoto, M.L., Teixeira, R.D.O. & Mantovani, M.S. (2001). *Averrhoa carambola L., Syzygium cumini (L) Skeels* and *Cissus sicyoides L.*: Medicinal herbal tea effects on vegetal and animal test systems.
39. Virk, B. S. and Sogi, D. S., (2004). Extraction and Characterization of Pectin from Apple (Malus Pumila. Cv Amri) Peel Waste. International Journal of Food Properties Vol. 7, No. 3, pp. 693–703, 2004.
40. Williams, K.J. and I. Tabas, 1995. The Response to Retention Hypothesis of Early Atherogenesis. Arterioscler. Thromb. Vasc. Biol., 15: 551-61.
41. Xu, Guangfei, Huang, X., Qiu, L., Wu, J. & Hu, Y. (2007).Mechanism Study of Chitosan on Lipid Metabolism in Hyperlipidemic Rats.Asia Pac J Clin Nutr;16 (Suppl 1):313-317.
42. Yang, B.K., Park, J.B. & Song, C.H. (2002). Hypolipidemic Effect of Exo-Polymer Produced in Submerged Mycelial Culture of Five Different Mushrooms.
43. Yap, C.F., Ho, C.W., Wan Aida, W.M., Chan, S.W., Lee, C.Y. & Leong, Y.S. (2009). Optimization of Extraction

Conditions of Total Phenolic Compounds from Star Fruit (*Averrhoa carambola* L.) Residues. Sains Malaysiana 38(4): 511–520

44. Yeh, Yen-Hung, Chen, M.H., Lee, Y.T., Hsieh, H.S. & Hwang, D.F. (2008). Effect of Taurine on Toxicity of Oxidized Cholesterol in Rats. Journal of Food and Drug Analysis, Vol. 16, No. 3, Pages 74-82.