

Modulatory Effects of some Fruit Juices on Lipid Profile in Rats Fed with High Lipid Diet

ABSTRACT

Background: Hyperlipidemia is a predisposing factor to several cardiovascular diseases, including atherosclerosis, myocardial infarction, heart attacks, and others. **Aim:** This study investigate the hypolipidemic properties of five selected fruits using rats pre-fed with High Lipid Diet (HLD) under experimental conditions. **Methods:** Raw fruit juices from Guava (*Psidium guajava*), Pawpaw (*Carica papaya*), Banana (*Musa acuminata*), Apple (*Pyrus malus*) and Pineapple (*Ananas comosus*) respectively, were administered orally at a dose of 7 ml/kg body weight for 14 days. Sera from the experimental rats were prepared for total cholesterol (TC), triglyceride (TG), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) assay, while Very Low Density Lipoprotein (VLDL) and Atherogenic Index (AI) were computed. **Results:** Feeding the experimental rats with High Lipid Diet increased their levels of TC, TG, LDL, VLDL and AI by 68.73%, 72.94%, 20.23%, 71.79% and 208.72% respectively with a corresponding decrease in HDL by 29.47%. Pineapple juice significantly reduced the levels of TC, TG, LDL, VLDL and AI by 84.44%, 69.39%, 95.19%, 70.15% and 96.99% respectively. Guava, banana and apple treated groups produced 56.72%, 23.88% and 07.46% increase in HDL respectively, with a non-significant ($p < 0.05$) value in pawpaw treated group, when compared to the HLD untreated control. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenes and steroids in all fruit juices and the absence of balsams and phenols. **Conclusion:** Consumption of the selected fruit juices improved lipid profile and reduced the risk associated with hyperlipidemia in experimental rats.

Keywords: Fruits, Hyperlipidemia, Lipid profile, Phytochemicals, Diet, Juice

1 INTRODUCTION

Guava (*Psidium guajava*), Pawpaw (*Carica papaya*), Banana (*Musa acuminata*), Apple (*Pyrus malus*) and Pineapple (*Ananas comosus*) are fruits

which are widely in use, they are also appreciated due to their roles in the treatment of various diseases [1].

Pineapple is a fruit of choice, which contains vitamin c, iron and other minerals; it's an effective laxative, a tonic and rejuvenative fruit [2]. Its juice from unripe fruit causes uterine contraction while that from the ripe fruit cures gastric irritability and helps in jaundice, the earlier is not recommended for pregnant women² because it may induce abortion or menstruation. Banana is another fruit of choice, which comes in a variety of sizes and colors to include yellow, purple and red. Its fruit is rich in vitamins and minerals.

Apple provides vitamins A and B, its high in carbohydrates and is an excellent source of dietary fibre [3]. Oral administration of apple juice has shown an insignificant change in body weight of experimental animals [4]. Aqueous and methanolic extracts from guava leaves have been reported to possess hypolipidemic activity in laboratory animals [5]. Guava and pawpaw fruits are edible for both human beings and animals and the juice are often used as a refreshing drink with no toxic effect [6,7]. Guavas are up to 5 times richer in vitamin C than oranges [8].

Fats, oils, and waxes are a group of naturally occurring organic materials call lipids, lipids are a constituent of plants or animals which are insoluble in water but soluble in organic solvents [9]. They are concentrated source of energy and structural components of cell. They also facilitate the intestinal absorption and transport of fat soluble vitamins A, D, E and K [9]. They cushion and protect some internal organs as the heart, kidneys and liver [9]. Excessive quantities or improper types of lipid-intake may result in hyperlipidemia, which is characterized by an abnormal elevation in one or more of the serum lipids such as Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and Triglycerides (TG). Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases including atherosclerosis, myocardial infarction, heart attacks, and cerebrovascular diseases [10]. Excess lipid in the body can also lead to obesity and other

adverse disease conditions. Obesity is a condition in which excess body fat accumulates, adversely affecting health [11].

Fruits generally have a high content of antioxidants, minerals, vitamins (Vit C) and phytochemicals that can act as antioxidants. They are often used in combinations as fruit salads. Gill [12] reported the beneficial effect of certain fruits to include apple, pear and peaches in cholesterol and LDL lowering effect in animal model. In the light of this, the aim of our study is to investigate the modulatory effects of raw fruit juices on the lipid profile of rats pre-fed with High Lipid Diet (HLD) under experimental conditions.

2 MATERIALS AND METHODS

2.1 Collection of plant materials

The plant materials; Guava (*Psidium guajava*), Pawpaw (*Carica papaya*), Banana (*Musa acuminata*), Apple (*Pyrus malus*) and Pineapple (*Ananas comosus*) used for this study were obtained from Jos, Plateau state and the fruits were identified in the Department of Plant Science and Technology, University of Jos before usage.

2.2 Experimental rats

The experimental animals (*Rattus norvegicus*) used for this study were obtained from the animal house of the University of Jos, Nigeria. Rats are of average weight 165 ± 18 g. Seven groups of four rats each were randomly distributed in cages and acclimatized for 7 days.

2.3 Administration of High Lipid Diet

High Lipid Diet was formulated by weighing a known amount of saturated fat (Margarine) into palletized rat chew (1:4 w/w) and mixing it uniformly [13-14]. This was fed to the test groups (groups 2-7) for four weeks prior to the start of the study and a total of six weeks experimentation period with water *ad libitum*.

2.4 Preparation of fruit juices

Briefly, the fruit peels were removed and discarded. The fruit its self were then separately blended using a blending machine. The crushed fruits were

poured into a clean white handkerchief (clothing material) and squeeze to get the juice extracts used in a clean, sterile container. The juice was then stored in the refrigerator at 15°C.

2.5 Treatment of experimental animals

Group 1 (Normal fed) and group 2 (HLD fed) received 1 ml of distilled water per day. Groups 3, 4, 5, 6 and 7(HLD fed) received 7 mL/kg bwt per day of Guava, Pawpaw, Banana, Apple and Pineapple respectively. The juice was orally administered once daily for a period of fourteen (14) days.

2.6 Collection of samples

At completion of the 14 days treatment, the rats were anesthetized at the time of sacrifice by being placed in a seal cotton wool soaked in diethyl ether inhalation jar. Blood samples were collected into centrifuge tubes and allowed to clot for about 45 minutes, after which they were spun at 3000 rpm for 5 minutes, the serum collected were transferred into bijou bottle using pasture pipette and kept for analysis.

2.7 Phytochemicals

Phytochemical tests were carried out using standard procedures described by Edeoga *et al.*, 2005 [15]. The tested phytochemicals include Alkaloid, Flavonoid, Tannins, Saponins, Balsam, Cardiac glycosides, Terpenes and Steroids, Resins and Phenol.

2.8 Lipid Profile Assay

Lipid profiles assayed include total cholesterol, triglyceride, HDL and LDL using standard procedure [16- 18].

The atherogenic index (AI) was calculated using the formula;

$$\text{Atherogenic Index (AI)} = \frac{\text{Total Cholesterol} - \text{HDL Cholesterol}}{\text{HDL cholesterol}}$$

Very Low Density Lipoprotein (VLDL) was calculated using the formula;

$$VLDL = \frac{\text{Triglyceride}}{2.2}$$

The percentage increase/decrease was calculated using the formula;

$$\% \text{ Decrease/Increase} = \frac{X_s - R_v}{R_v} \times 100$$

Where

X_s = Sample value, R_v = Reference value (Control or HLD).

2.9 Statistical analysis

Data were presented as Mean \pm SD of 4 replicates and were analyzed using DMRT following one-way analysis of variance (ANOVA) using SPSS 20.0 computer software package (SPSS Inc., Chicago, U.S.A) where applicable. Differences at $p < 0.05$ were considered significant.

3 RESULTS

Raw juice from all fruits significantly ($p < 0.05$) decrease the levels of total cholesterol, TG VLDL and LDL when compared to the HLD control. Guava juice showed a significant increase ($p < 0.05$) in HDL when compared to HLD while all other fruits could not express a similar effect, however, they were able to prevent a significant decrease in the lipoprotein (Table 1). All juice improved the AI compared to HLD untreated (Figs. 1-2).

Phytochemical screening of the juice from the various fruits in the study revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenes and steroids in all juice and the absence of balsams and phenols. On the other hand, resins were detected in only pineapple while tannins were absent in pawpaw and pineapple (Table 2).

Table 1: Effect of Raw Fruit Juice on Lipid Profile in Hyperlipidemic Rats

Lipid Profile (mmol/L)

Groups	TOTAL CHOL	TRIG	HDL	LDL	VLDL
Control	2.59 \pm 0.22	0.85 \pm 0.04	0.95 \pm 0.04	1.73 \pm 0.16	0.39 \pm 0.03
HLD	4.37 \pm 0.37 (\uparrow 68.73 [#])	1.47 \pm 0.13 (\uparrow 72.94 [#])	0.67 \pm 0.15 (\downarrow 29.47 [#])	2.08 \pm 0.16 (\uparrow 20.23 [#])	0.67 \pm 0.08(\uparrow 71.79 [#])
Guava	1.48 \pm 0.17 ^b (\downarrow 66.13 [~])	0.96 \pm 0.10 ^b (\downarrow 35.69 [~])	1.05 \pm 0.11 ^c (\uparrow 56.72 [~])	0.41 \pm 0.08 ^b (\downarrow 80.29 [~])	0.44 \pm 0.04 ^b (\downarrow 34.33 [~])
Pawpaw	1.07 \pm 0.13 ^b (\downarrow 75.51 [~])	0.55 \pm 0.11 ^b (\downarrow 62.59 [~])	0.62 \pm 0.14 ^a (\downarrow 07.46 [~])	0.47 \pm 0.09 ^b (\downarrow 77.40 [~])	0.25 \pm 0.04 ^b (\downarrow 62.69 [~])
Banana	1.32 \pm 0.17 ^b (\downarrow 69.79 [~])	0.82 \pm 0.25 ^b (\downarrow 44.22 [~])	0.83 \pm 0.12 ^a (\uparrow 23.88 [~])	0.45 \pm 0.07 ^b (\downarrow 78.37 [~])	0.37 \pm 0.10 ^b (\downarrow 44.78 [~])
Apple	1.22 \pm 0.17 ^b (\downarrow 72.08 [~])	0.62 \pm 0.14 ^b (\downarrow 57.82 [~])	0.72 \pm 0.14 ^a (\uparrow 07.46 [~])	0.10 \pm 0.01 ^b (\downarrow 95.19 [~])	0.28 \pm 0.06 ^b (\downarrow 58.21 [~])
Pineapple	0.68 \pm 0.18 ^b (\downarrow 84.44 [~])	0.45 \pm 0.12 ^b (\downarrow 69.39 [~])	0.60 \pm 0.14 ^a (\downarrow 10.44 [~])	0.10 \pm 0.01 ^b (\downarrow 95.19 [~])	0.20 \pm 0.03 ^b (\downarrow 70.15 [~])

Values are mean \pm SD (n=4)
 a = statistically non-significant ($p < 0.05$) when compared to HLD
 b = significantly decreased ($p < 0.05$) when compared to HLD
 c = significantly increased ($p < 0.05$) when compared to HLD
 Values in brackets are % decrease (\downarrow)/increase (\uparrow) where # when compared to normal control, * when compared to HLD

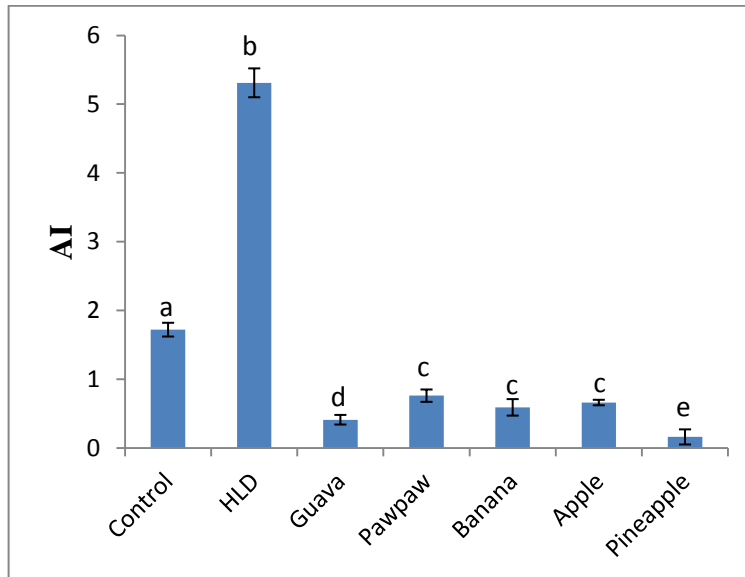


Fig. 1: Atherogenic index of different fruit juice after 14 days administration

Values are mean \pm SD (n=4)
 Values with different superscript are significantly different ($p < 0.05$)
 b = significantly increased ($p < 0.05$) when compared to control
 c,d,e = significantly decreased ($p < 0.05$) when compared to control and HLD

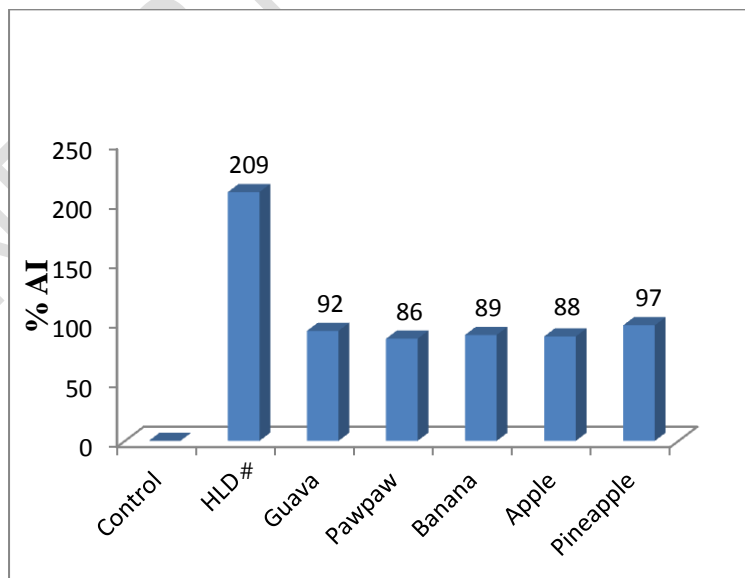


Fig. 2: Percentage atherogenic index of different fruit juice after 14 days administration

% are approximated to the nearest whole number, where # when compared to normal control against others compared to HLD

Table 2: Phytochemicals Detected in Raw Fruit Juices

Phytochemicals	Guava	Pawpaw	Banana	Apple	Pineapple
Alkaloid	+	+	+	+	+
Flavonoid	+	+	+	+	+
Tannins	+	-	+	+	-
Saponins	+	+	+	+	+
Balsam	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+
Terpenes and Steroids	+	+	+	+	+
Resins	-	-	-	-	+
Phenol	-	-	-	-	-

Note: + = Detected; - = Not detected

4 DISCUSSIONS

A significant amount of phytochemicals was detected in all fruit juice. Balsam, resins and phenols were however not detected in the fresh samples. Presence of these phytoconstituents and their ability to lower total cholesterol or affect its metabolism may be of great benefit to fruit consumers. Plant sterols (β -sitosterol) have been reported to possess cholesterol – lowering activity [19]. Phytosterol are reported to displace intestinal cholesterol and reduce cholesterol absorption from the intestine [20]. The esterification of phytosterols is also an important advantage, it allowed the major site of cholesterol absorption (the small intestine) to have an enhance delivery of sterols [21]. Saponins are found to inhibit the enterohepatic circulation of bile acid, making it unavailable for intestinal absorption, which leads to decrease cholesterol absorption from the intestine [22]. Terpenes, steroids and

saponins were constituents of all the fruit juice considered in the present study.

Flavonoids act as antioxidants, protecting LDL cholesterol from oxidation, inhibit platelet aggregation and acting as an anti-inflammatory and anti-tumor agents [23-25]. Xie *et al.* [26] has reported that extracts enriched in flavonoids such as *Ananas comosus* L. (Pineapple) leave inhibit HMG-CoA reductase activity, the rate limiting enzyme in the biosynthesis of cholesterol. HLD causes marked hypercholesterolemia; increased levels of TC, TG, LDL-C and VLDL-C. Elevated lipid levels specially hypercholesterolemia results due to increased absorption in the gut or endogenous synthesis [27]. Feeding the experimental animals with HLD increased their levels of Total cholesterol, triglyceride, LDL and AI by 68.73%, 72.94%, 20.23% and 208.72% respectively with a corresponding decrease in HDL (good cholesterol) by 29.47%. Pineapple juice significantly reduced the levels of total cholesterol, triglyceride, LDL and AI by 84.44%, 69.39%, 95.19% and 96.99% respectively.

The effects of regular feeding of *Pyrus malus* (apple) whole fruit and its juice on total serum cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and phospholipids have been studied in normal albino rabbits [4]. It was observed that the whole fruit as well as juice caused significant and progressive decrease in total serum cholesterol, LDL cholesterol and triglycerides within a period of 30 days [4]. On the other hand, serum phospholipids and HDL cholesterol showed progressive and significant increase [4]. Their studies also showed that the whole fruit was better than its juice in improving the lipid profile.

Although, the juice (pawpaw, banana, apple and pineapple) could not significantly ($p < 0.05$) increase the level of serum HDL in experimental rats when compared to guava, they prevented a significant decrease in the level

of the serum lipoprotein. Guava, banana and apple produce 56.72%, 23.88% and 07.46% increase in HDL respectively, when compared to the HLD untreated control. Elevated levels of LDL-C, triacylglycerol and total cholesterol with reduced HDL-C and High levels of fat in the form of cholesterol will enhance the development of atherosclerosis, related cerebrovascular disorders as well as other peripheral vascular disease [28-29]. Juice extracts from these fruits could be of great benefit in prevention of these diseases associated with lipid metabolism.

5 CONCLUSION

Conclusively, the results obtained from the study shows that fruit juice from Guava (*Psidium guajava*), Pawpaw (*Carica papaya*), Banana (*Musa acuminata*), Apple (*Pyrus malus*) and Pineapple (*Ananas comosus*) possess potent hypolipidemic properties on blood cholesterol level of experimental rats. Flavonoids, saponins, steroids and other phytochemicals detected may play beneficial role in these effects.

ETHICAL APPROVAL

Ethical clearance was approved by the University of Jos Ethical Committee with reference number UJ/FPS/F17-00379.

REFERENCES

1. Rajeev S. Improve your health with apple, Guava, Mango. Diamond Pocket Books (P) Ltd, 2005; pp22: 88-121.
2. Deneo-pellegrini H, De Stefani E, Ronco A. Vegetables, fruits, and risk of colorectal cancer: a case-control study from Uruguay. *Nutrition & Cancer* 2006; 25(3): 297-304.
3. Ferree DC and Warrington J. Apples: Botany, Production and Uses. CABI Publishing 2000.
4. Sangeeta A, Pachori SB, Pandey DN and Pant MC. Effects of feeding *Pyrus malus* (Apple) on serum lipid profile with special reference to high and low density lipoprotein cholesterol levels in normal albino rabbits. *Indian Journal of Clinical Biochemistry* 1990; 5(2): 91-94.

5. Shinde S, Chivate N, Kulkarni P, and Naikwade N. Hypolipidemic Activity of *Psidium Guajava Linn* Leaves Extracts in Hyperlipidemic Rats, International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(1): 70-72.
6. Emeruwa AC. Antibacterial substance from *Carica papaya* fruit extract. J. Nat. Prod., 1982; 45(2): 123–127.
7. Iwu MM., Handbook of African Medicinal Plants. CRC Press 1993.
8. Conway P. Tree Medicine – a comprehensive guide to the healing power of over 170 trees. Judy Piatkus (Publishers) Ltd. 2001.
9. Fahy E, Subramaniam S, Murphy R., Nishijima M, Raetaz C, Shimizu T, Spener F, Van Meer G, Wakelam M and Dennis EA. Update of the LIPID MAPS comprehensive classification system for Lipids. Journal of Lipid Research 2009; 50: S9-S14.
10. Na YY, Hyeung RK, Hae YC, Jae SC. Antihyperlipidemic effect of an Edible brown algae, *Ecloniastolonifera*, and its constituents on Poloxamer 407- Induced Hyperlipidemic and Cholesterol-fed rats. Archives of pharmacal research 2008; 31 (12):1564-71.
11. Barness LA, Opitz JM, Gilbert-Barness E. Obesity: genetic, molecular, and environmental aspects. Am. J. Med. Genet.2007;A143A (24): 3016-34.
12. Gill LS. *Carica papaya* L. In: Ethnomedicinal uses of plants in Nigeria. Benin City: UNIBEN Press2002; 57-58.
13. Luka CD, Idoko KM. And Jawonisi IO., Effect of Honey on Albino Rats fed with High Lipid Diet, Journal of Medicine in the Tropics 2010; 12: 33-36.
14. Tijjani H. and Luka CD. Effects of *Afromomum melegueta*, *Zingiber officinale* and *Piper nigrum* Some Biochemical and Haematological Parameters in Rats Fed with High Lipid Diet, *Int. J. Pure App. Biosci.*2013;1(3): 61-67.

15. Edeoga HO, Okwa DE, and Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol.* 2005; 4(7): 685-688.
16. Hainline A, Cooper GR and Olansky AS. CDC survey of high density lipoprotein cholesterol measurement: A report, Center for Disease Control publication, Atlanta, GA. 1980.
17. Fredrickson DS, Levy RI, and Lees RS. Fat transport in lipoproteins-An integrated approach to mechanisms and disorders, *New England Journal of Medicine* 1967; 276: 148 –156.
18. Albers JJ, Warmick GR and Cheung MC. Quantitation of high density lipoproteins. *Lipids* 1978; 13: 926-932.
19. Farquhar JW, Smith RE and Dempsey ME. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. *Circulation* 2006; 14:77-82.
20. Ikeda I, and Sugano M. Inhibition of cholesterol absorption by plant sterols for mass Intervention. *Current Opinion Lipidol* 1998; 9:527-531.
21. Mattson FH, Grundy SM, and Crouse JR. Optimizing the effect of plant sterols on cholesterol absorption in man. *Am. J. Nutr.* 2002; 35:697-700.
22. Fuhrman B, Volkora N, Kaplan M, Pressor D, Aitias J, Hayek T, and Aviram M. *Nutrition* 2002; 18: 268-275.
23. Smith TJ and Yang CS. Effects of food phytochemicals or xenobiotic metabolism. In 'Food Phytochemicals for Cancer Prevention I. Fruits and Vegetables' (MT Hauang, T Osawa, CT Ho, and RT Rosen, eds), 2004; 17-48.
24. Cook NC. and Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutr. Biochem.* 2006; 7: 66-76.

25. Manach C, Regerat F, and Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutr. Res.* 2006;16: 517-544.
26. Xie W, Wang W, Su H, Xing D, Cai G, Du L. Hypolipidemic mechanisms of *Ananas comosus* L. leaves in mice: Different from fibrates but similar to statins. *J Pharmacol Sci.* 2007;103: 267–74.
27. Vilasinee H, Anocha U, Noppawan PM, Nuntavan B, Hitoshi S, Angkana H, Chuthamane S. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethnopharmacology* 2006;103(2): 252-260.
28. Carr AC, Zhu B and Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α -tocopherol (vitamin E). *Circulation Research* 2000;87:349–354.
29. Howard BV, Van Horn L, Hsia J, *et al.* Low-fat dietary pattern and risk of cardiovascular disease: the women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA: The Journal of the American Medical Association* 2006; 295 (6): 655-66.