

# ANTI-HYPERLIPIDEMIC ACTIVITY OF *Persea americana* PEEL EXTRACTS AGAINST HIGH-FAT DIET IN RATS

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## Abstract:

Hypercholesterolemia is a dominant risk factor for atherosclerosis and cardiovascular diseases. It is now widely accepted that atherosclerosis is a complex multicellular process involving oxidation of cholesterol and to intracellular accumulation. This accumulation causes a cascade of inflammatory processes, resulting in an unstable atherosclerotic plaque causing myocardial infarction. From ancient times, botanicals have played a major role in the lifestyle of people. The association of hyperlipidemia with the development of atherosclerotic lesion has promoted widespread search for plant based compounds which effectively control the lipid profile in the blood and tissues with least or no toxic effect. *Persea americana* (Butter Fruit) is a popular superfood owing to its nutritional values. However, critical data of *Persea americana* is limited on their effects. The present study is aimed at evaluating the efficacy of *Persea americana* methanolic peel extracts (PAMPE) for its antioxidant & antihyperlipidemic properties.

The PAMPE contains an array of phytochemicals phenols, flavonoids, terpenoids etc., however flavonoids were on a higher scale. Further the antioxidant potential was demonstrated with a battery of in vitro systems (DPPH, ABTS). Further male albino rats were supplemented with PAMPE (25-140mg/kg bw/day for 2 weeks). Serum profile showed that there was marked reduction in TG, TC & LDL among PAMPE rats in a time & dosage dependent manner. HDL levels were significantly improved with PAMPE oral supplement, In addition total serum cholesterol levels were reduced among rats receiving PAMPE supplements. Our result indicate a potent hypocholesterolemic effect of PAMPE in vivo & Support the therapeutic candidacies. However assessment of molecular mechanisms of PAMPE is warranted.

**Keywords :** Atherosclerosis, hyperlipidemia, herbal drugs, lipids.

## 1. Introduction

Diseases of the cardiovascular system are the most common cause of death. Lifestyle changes have a significant impact on the health of the people. The modernization of societies appears to result in a dietary pattern that is high in saturated fats and refined sugars and is low in fiber content [1]. It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. High levels of low-density lipoprotein (LDL) cholesterol accumulate in the extracellular sub endothelial space of arteries; these are highly atherogenic and toxic to vascular cells, leading to atherosclerosis, hypertension, obesity, diabetes, and functional depression in organs such as the liver, heart, and kidneys [3]. Clinical trials have shown that lowering lipids reduces the morbidity and mortality associated with cardiovascular complications [4]. Intensive reductions of LDL-cholesterol levels have also been found to reverse atherosclerosis and decrease the progression of cardiovascular disease [5, 6].

Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the etiology of several diseases, including atherosclerosis and coronary heart disease [7, 8]. Oxidative stress contributes to the development of atherosclerosis in the vascular wall through the formation of ROS [6]. Increased formation of free radicals is accompanied by perturbations in antioxidant status, resulting in oxidative damage to cellular components [8]. Hyperlipidemia is reported to be associated with the oxidative stress that results from the increased production of ROS or impairment of the antioxidant system [9].

Many antihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezetimibe etc reduce cholesterol level with different condition [6]. Currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [7]. Since ancient times plants have been exemplary sources of medicine. Plants still constitute one of the major raw materials in drugs for treating various ailments of human being, although there has been significant development in the fields of synthetic drug chemistry and antibiotics. During the last two decades, considerable changes have taken place in the medicinal system all over the world. Because of the general awareness of the widespread toxicity and harmful after-effects associated with the long term use of synthetic drugs and antibiotics, drugs from natural sources are being preferred. According to the World Health Organization [8], a medicinal plant is any plant in which, one or more of its organs contains substances that can be used for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutics properties and constituents

have been established scientifically from plants that are regarded as medicinal but which have not yet been subjected to thorough investigation.

*Persea americana* commonly known as Avacado is a plant species belonging to Lauraceae family. The cultivators of West Indian race are localized folks in Maharashtra, Tamil nadu and Karnataka. They are well known among the people and widely used for their nutritive and medicinal properties; they are rich source of poly phenolic compounds and phenolic acids. Avocado contains one to two times more protein than any other fruit, is high in manganese, phosphorous, iron and potassium, but low in sodium, and also contains vitamin E, vitamin C,  $\beta$ -carotene, thiamin, riboflavin, nicotinic acid and folate (Rainey et al, 1994). Avocado is rich in oil (15% g/100 g fresh fruit) that is mainly monounsaturated (Willis et al, 1986) and is a good source of the essential linoleic acid (Bergh, 1992). The amount of simple sugars in the avocado fruit is low, but in contrast, it contains appreciable levels of dietary fiber (DF) and is the highest in fiber among fruits (Bergh, 1992). Avocado contains several structural polysaccharides, including insoluble (cellulose and lignin) and soluble (hemicelluloses and pectin) DF (Sanchez-Castillo et al., 1995). DF impose positive modifications on viscosity, motility, nutrient absorption, content, transit time, emptying, and probiotic properties of the entire digestive track (Kritchevsky and Bonfield, 1995). These modifications may resolve constipation, reduce fat absorption, lower glycemic index and plasma insulin levels, alter colon fermentation and microbial proliferation, and reduce plasma cholesterol (Kritchevsky and Bonfield, 1995). Therefore, adding recommended levels of DF to the diet is considered vital for normal intestine performance, good health, and for controlling major risk factors for diabetes, obesity, gallstones, hypercholesterolemia and heart disease

Even though the research has been extensively done and documented on the whole fruit, seed, leaf, pulp of *Persea americana* and shows a promising herbal medicine to hyperglycemia & Hyperlipidemia. There is no such scientific scrutiny done on the "PEEL" of *Persea americana*, which is usually discarded as waste, This has nurtured research interest in evaluating peel of *Persea americana* for its antioxidant-rich traditional remedies and alternative medicines as potentially efficacious cholesterol-lowering therapies which have few, or no, side-effects.

## 2. Materials and methods

The fresh *Persea americana* fruits were obtained from the local markets of wayanad district, Kerala, which is a West Indian race.

**Preparation of extract:** 23.25 grams of peel were scraped of fresh *Persea americana* fruit and were dried in hot air oven for 2 days making sure the temperature not exceeding 40°C, and then the samples were grounded to obtain powder. The sequential Extraction of *Persea americana* was done at room temperature starting with non polar solvents to polar solvents (hexane, chloroform-ethyl acetate, methanol, water) respectively. About 2g of dried sample was soaked in respective solvents overnight on magnetic stirrer. Subsequently using the solvent methanol, the solvent extraction is done by soxhlet apparatus, the final extract were concentrated on vacuum and stored at 4°C for further antioxidant & antihyperlipidemic assays.

### Free Radical Scavenging Activity: DPPH Assay

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution, 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below: A-B Percent (%) inhibition of DPPH activity =  $(A-B) / A \times 100$  Where A = optical density of the blank and B = optical density of the sample.

### ABTS Assay [2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulphonic Acid)] Free Radical Scavenging Activity Assay:

The ABTS+ stock solution was prepared by reacting ABTS (Sigma Aldrich, India) aqueous solution (7 mM) with 2.45 mM aqueous solution of potassium persulfate (Merck, India) in equal quantities; the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. The working solution of ABTS+ was obtained by diluting the stock solution in methanol to give an absorbance of  $0.70 \pm 0.02$  at 734 nm. Then, 2.0 mL of ABTS+ solution was mixed with 1 mL of the aqueous extracts at different concentrations (0.5-5.0 mg/mL). The mixture was then incubated at room temperature for exactly 10 min in the dark. The control was prepared by mixing 2.0 mL of ABTS+ solution with 1 mL of double distilled water. The absorbance was measured against a blank at 734 nm using spectrophotometer (Systronics Visiscan 167). BHT (Merck, India) was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on ABTS+ was calculated as % inhibition (1%) using the following equation:  $1\% = [(A_0 - A_s) / A_0] \times 100$  Where  $A_0$  is the absorption of control and  $A_s$  is the absorption of the tested extract

**Animals:** Normal healthy male Wistar Albino rats (180- 240g) were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12: 12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum. The study was approved by the animal ethics committee of ( FCP/EC-5/273/2014-15).

**Development of high fat diet fed rats:** The total days considered for the present study is for 45 days, firstly the rats were acclimatized, prior the experimental treatments, their body weights were measure before the start of the experiment Rat was fed with two dietary regimes such as Normal pellet Diet (NPD) and High fat Diet (HFD). The rat was feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks Rats were tested for acute toxicity test, screened for the presence of total serum cholesterol prior the screening the rats

were starved for 6 hours. The rats showing the presence high cholesterol were subjected with methanolic peel extracts of *Persea americana* and the same experimental design documented below. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages. The studies were conducted in the four groups of six animals each

- **Group I:** Normal control without any treatment(NFD).
- **Group II:** HFD control(High Fat Diet) for 2 weeks
- **Group III:** Administered with HFD followed by methanolic peel extracts of *Persea americana* at an optimum dosage of 150 mg/kg body wt. for 2 weeks
- **Group IV:** Served as positive control and received atorvastatin (1.2 mg/kg body weight).

The extracts as well as atorvastatin were suspended in 2% tween 80 (Satheesh and Kottai, 2012) and fed to the respective rats via oral intubation. The animals were sacrificed at the end of experimental period. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes and the following estimations were carried out enzymatically. Serum total cholesterol (TC), total triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were enzymatically analyzed.

#### Statistical analysis

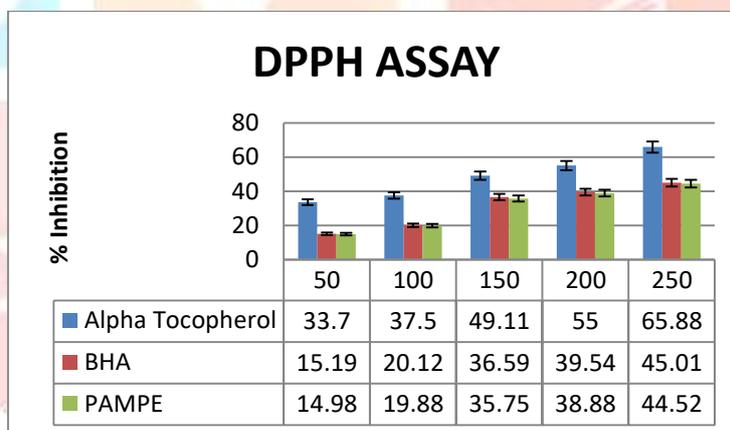
One way analysis of variance (ANOVA) followed by Dunnett's t-test was carried out and  $P < 0.005$  was considered significant.

#### 3. Results :

*Persea americana* peel sample extracted sequentially with hexane, ethyl acetate, water, methanol, acetone, chloroform & ethanol. were subjected to qualitative phytochemical screening which provides the essential information regarding the chemical constituents and the results are interpreted as follows: Quantitative tests of the extract show significant indication about the presence of metabolites like Phenols, Flavanoids, Terpenoids, steroids in abundance, thus reflecting its importance. The preliminary phytochemical screening of this plant crude extracts showed the maximum phytochemical constituents with methanolic extracts and hence for further in vitro studies the methanolic extracts were considered

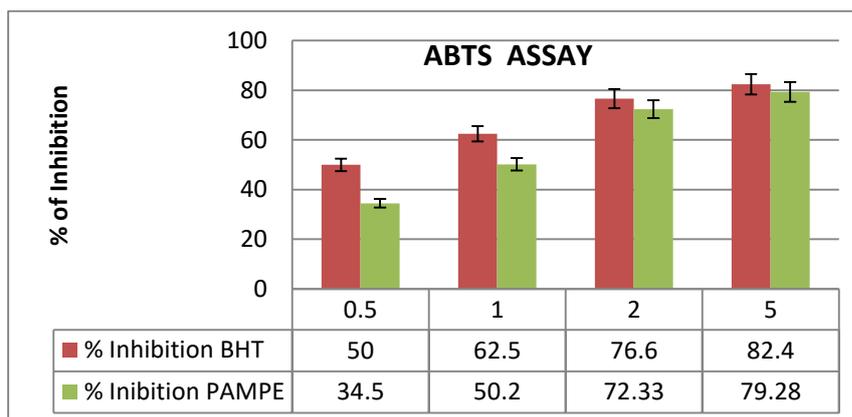
#### Free Radical Scavenging Activity: DPPH Assay

The essence of DPPH method is that the antioxidants react with the stable free radical ie  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl (deep violet color) and convert it to a  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazine with discoloration. The degree of discoloration indicates the scavenging potential of the sample antioxidant



The scavenging activity of methanolic peel extract of *Persea americana* was 45% at 250  $\mu\text{g/ml}$  concentration. The BHA and  $\alpha$ -Tocopherol standards were also carried out which showed 45.01% and 65.88% at 250  $\mu\text{g/ml}$  concentration respectively. Thus these results indicate the outstanding scavenging effects on DPPH.

#### ABTS Assay



The highest % inhibition value was found to be 79.28% at 5.0 mg/mL for peel methanolic extract comparable to standard 82.4%. In general, fractions with high phenolic content showed high radical scavenging and antioxidant activity. Thus indicating small and high molecular mass phenolics including flavanoids, phenolic acids, terpenoids and tannins as good quenchers of free radicals.

#### Analysis of antihyperlipidemic effect:

Preliminary screening test was performed at dosage of 50, 100, 150, 200, 250 mg/kg body weight. The methanol peel extract was tested for hypolipidemic effect in the albino rats at the selected optimum dosage of 140 mg/kg body weight and administered orally.

Parameters tested	Group I Normal control	Group II HFD control	Group III HFD + PAMPE	Group IV Positive control
Total Cholesterol(mg/dl)	77.77± 4.05	152 ± 2.48	87.45 ± 1.65	31.47± 2.54
Triglycerides(mg/dl)	70.15± 6.16	154.14±2.17	98.33±2.45	34.06±2.89
HDL cholesterol(mg/dl)	34.81± 1.58	22.59±0.88	31.17±1.51	51.80± 3.97
LDL cholesterol(mg/dl)	27.08± 2.91	56.04±2.11	41.41± 2.81	97.22± 5.28

One way analysis of variance (ANOVA) followed by Dunnett's t-test was carried out and  $P < 0.005$ , the test result is considered significant.

Serum profile showed that there was marked reduction in TG, TC & LDL among PAMPE rats in a time & dosage dependent manner. HDL levels were significantly improved with PAMPE oral supplement, In addition total serum cholesterol levels were reduced among rats receiving PAMPE supplements.

#### 4. Discussion:

The present investigation was undertaken to assess the anti-oxidant and antihyperlipidemic activity of *Persea americana methanolic peel extracts*. Several epidemiological studies have shown flavonoid intake is associated with a low risk of cardiovascular disease (Marjorie et al., 2012). Our results indicated that the phytochemical constituents of PAMPE may play an important role in its antioxidant and anti-hyperlipidemic activity.

In the present study, feeding rats with diets rich in cholesterol resulted in increased TC, TG and LDL cholesterol levels. This model was used to study the potential of hypolipidemic effect of supplementations of PAMPE that contained significant amounts of antioxidants properties. From this study, we found that daily oral administration PAMPE supplements shows a positive result on significantly reduced total cholesterol levels in plasma after 2 weeks of supplementation. This result agrees with literature where depleted level of HFD fed hyperlipidemia. HDL is directly anti-androgenic and it is believed to remove cholesterol from the developing lesions. LDL is a risk factor and plays a role in several steps of atherosclerosis. A decrease in oxidative stress and protection of LDL from oxidation might therefore be a strategy with great promise for prevention of atherosclerosis associated cardiovascular disease. The intense interest in this area results in part from the generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering regimes.

In conclusion, it could be said that PAMPE exhibit a potent hyperlipidemic effect in vivo & Support the therapeutic candidacies. However assessment of molecular mechanisms of PAMPE is warranted.

#### Conflict of interest statement:

We declare that we have no conflict of interest.

#### Acknowledgement:

The author's thank Director, Pooja Bhagavat Memorial Mahajana Education Centre Post Graduate wing of SBRR Mahajana First Grade College, Mysore and Mahajana Education Society, Mysore for their constant encouragement and support.

#### 5. References:

- Brai BIC, Odebolt MA, Agoma PU. Effect of Persia Americana leaf extract on body weight and liver lipids in rats fed with hyperlipidemic diet. *Afr J Biotechnol* 2007; 16(8): 1007-1111.
- K. S. Jain, R. R. Kulkarni, and D. P. Jain, "Current drug targets for antihyperlipidemic therapy," *Mini Reviews in Medicinal Chemistry*, vol. 10, no. 3, pp. 232–262, 2010.
- A. L. Amundsen, L. Ose, M. S. Nenseter, and F. Y. Ntanios, "Plant sterol ester-enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia," *American Journal of Clinical Nutrition*, vol. 76, no. 2, pp. 338– 344, 2002.
- T. Ichihashi, M. Izawa, K. Miyata, T. Mizui, K. Hirano, and Y. Takagishi, "Mechanism of hypocholesterolemic action of S- 8921 in rats: S-8921 inhibits ileal bile acid absorption," *Journal of Pharmacology and Experimental Therapeutics*, vol. 284, no. 1, pp. 43–50, 1998.

- W. Shi, M. E. Haberland, M.-L. Jien, D.M. Shih, and A. J. Lusis, "Endothelial responses to oxidized lipoproteins determine genetic susceptibility to atherosclerosis in mice," *Circulation*, vol. 102, no. 1, pp. 75–81, 2000.
- D. L. Tribble, "Antioxidant consumption and risk of coronary heart disease: emphasis on vitamin C, vitamin E, and  $\beta$ -carotene: a statement for healthcare professionals from the American Heart Association," *Circulation*, vol. 99, no. 4, pp. 591–595, 1999.
- E. Hopps, D. Noto, G. Caimi, and M. R. Averna, "A novel component of the metabolic syndrome: the oxidative stress," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 20, no. 1, pp. 72–77, 2010.
- M.M.M. F. Duarte, J.B.T.Rocha, R.N.Moresco et al., "Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia," *Clinical Biochemistry*, vol. 42, no. 7-8, pp. 666–671, 2009.
- Aida Zarfeshani<sup>1</sup>, Mohd Sokhini Abd Mutalib<sup>1</sup> and Huzwah Khaza' ai<sup>2</sup> **Evaluating of High Fructose Diet to Induce Hyperglycemia and its Inflammatory Complications in Rats** Pakistan Journal of Nutrition 11 (1): 21-26, 2012.
- D. Eze, I.A. Mohammed, K.Y. Musa, Y. Tanko and A.S. Isa **Effect of Ethanolic Leaf Extract of Mucunapruriens (fabaceae) on Lipid Profile in Alloxan-Induced Diabetic Wistar rats** British Journal of Pharmacology and Toxicology 3(3): 102-109, 2012
- Manal M.S I .M. Shehata and S.S.A. Soltan **Effects of Bioactive Component of Kiwi Fruit and Avocado (Fruit and Seed) on Hypercholesterolemic Rats** World Journal of Dairy & Food Sciences 8 (1): 82-93, 2013
- Smitha Grace S. R\*, Jyoti Bala Chauhan and Chaithra Ratnakar Jain. In Vitro Alpha Amylase Inhibitory Effect And Antioxidant Activity By Peel And Seed Extracts of Persea Americana , World journal of pharmaceutical and Life science, 2016; 2(3): 261-269.
- Smitha Grace S. R\*, Jyoti Bala Chauhan and Chaithra Ratnakar Jain. Preliminary phytochemical investigation and TLC analysis of peel and seed extracts of Persea americana ,Asian Journal of Pharmaceutical Science & Technology, 2015; 5(3): 167-171.

