Effect of hydroalcoholic fruit extract of *Persea americana* Mill. on high fat diet induced obesity: A dose response study in rats

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The fruits of *Persea Americana* Mill., commonly known as Avocado, are traditionally consumed for various health benefits including weight reduction. Here, we studied the effect of hydroalcoholic fruit extract of *Persea americana* (HAEPA) on high fat diet (HFD) induced obesity in rats. Obesity was induced in male Sprague Dawley rats by feeding HFD for 14 wk. The hypolipidemic effect was evaluated by co-administering 25, 50, 100 and 200 mg/kg body wt. of HAEPA. There was a significant increase in weight gain, body mass index (BMI), blood lipids, low density lipoproteins (LDL), lipid peroxides (LPO) and serum transaminases in HFD fed rats. HFD+HAEPA fed rats showed a significant decrease in blood lipids, LPO, liver lipids and increase in antioxidant status when compared to HFD control rats. The activity of lipid metabolic key enzymes such as fatty acid synthase and HMG CoA reductase in liver were also found to be decreased significantly in HAEPA co-administered rats. Lipoprotein lipase activity was found increased in HFD+HAEPA rats. Among the 4 doses studied, 100 mg of HAEPA/kg body wt. exhibited optimum hypolipidemic activity. Histopathological observations in liver and visceral adipose tissue added more evidence for the lipid lowering effect of HAEPA. It can be concluded that avocado fruit extract can act as hypolipidemic agent probably by modulating the activities of HMG CoA reductase and fatty acid synthase in liver.

**Keywords:** Alligator pear fruit, Avocados, Blood lipids, BMI, Butter fruit, HFD, HMG CoA reductase, Hyperlipidemia

Obesity is a multifactorial disorder characterized by excess fat in adipose tissues due to unhealthy dietary practices leading to an imbalance between energy intake and energy expenditure\(^1\). Obesity is also associated with insulin resistance, hyperlipidemia and hypertension. High fat diet (HFD) intake often claimed as responsible for the increase in adiposity. Studies have shown that HFD can easily induce human and experimental obesity\(^2,5\).

Hydroxymethyl glutaryl (HMG) CoA reductase is a rate limiting enzyme of cholesterol biosynthesis\(^3,6\). This enzyme is the target of widely available hypolipidemic drugs such as statins\(^3,6\). Fatty acid synthase (FAS) exist as multi-enzyme complex which contains seven enzymes including thioesterase. HFD provides precursors for endogenous fatty acid biosynthesis where fatty acid synthase is upregulated\(^7\). Hepatic lipoprotein lipase (LPL) hydrolyses triacylglycerol and downregulates during abnormal lipid metabolism\(^8\). Hypolipidemic drugs are usually tested for their modulating effect on the above mentioned key enzymes.

Reactive oxygen species (ROS) formed in cells are detoxified by acting on enzymatic and non-enzymatic antioxidants which otherwise cause oxidative stress and may damage biomembranes, lipids, lipoproteins and nucleic acids\(^3,9,10\). Oxidative stress caused by ROS also associated with the pathogenesis of obesity due to hyperlipidemia\(^6\). Oxidation of LDL plays an important role in causing cardiovascular diseases\(^6\).

Currently, “statin” group of drugs is about choice for lowering cholesterol especially LDL cholesterol\(^6,11\). Due to adverse side effects of cholesterol lowering drugs various natural products including crude extracts and isolated compounds widely used traditionally and the research is focused on evaluating the hypolipidemic potential of various plant products\(^3,5,12,13\).

The fruits of Avocado tree (*Persea americana* Mill., Lauraceae), commonly known as butter fruit or alligator pear fruit, are native to Central America (Mexico, Guatemala, Antilles) and showed easy adaptation in other tropical regions. Avocado fruits are rich in vitamin A (beta carotene), C and E, the natural antioxidants which protect the cells from the harmful effects of “free radicals”\(^14\). Avocado fruit extract possess anti-proliferative property when tested.
in human cancer cell lines. Avocados are rich in phytochemicals that have antidiabetic, antioxidant, antimicrobial, antivenom and chemopreventive properties. Avocados contain one to two times more protein than any other fruits and high in manganese, phosphorous, iron and potassium, but low in sodium, and are rich source of monounsaturated fatty acids (MUFA).

In the present study, we assessed the lipid lowering effect of hydroalcoholic fruit extract of *Persea americana* (HAEPA) using rat model of HFD induced obesity.

**Materials and Methods**

**Chemicals and reagents**

All chemicals and solvents used for the analysis were of analytical grade bought from Excel Chemicals and Scientific Advance Company, Chennai through authorized dealers.

**HAEPA preparation**

Fresh avocados were collected from fruit shops and authenticated by the plant taxonomist Dr P Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai (PARC/2013/1458). The edible portion was chopped into small pieces, finely minced and repeatedly extracted with 70% ethanol. The extract was concentrated using rotary vacuum evaporator and lyophilized. The yield was 10.45 g/100 g.

**Preparation of high fat diet (HFD)**

High fat diet (HFD) was prepared and fed to the experimental animals as recommended by Nascimento et al. The ingredients used to prepare HFD [ground labina, roasted peanuts, casein, corn oil and French fried potatoes] were ground and then mixed with vitamins and minerals (1-1.2 g per 100 g of HFD). The mixture was then made into pellets and dried in a ventilated drying oven at 55±5°C. The weight of the lipid residue was determined.

**Biochemical analyses**

**Estimation of blood lipids and liver marker enzymes**

The plasma was assayed for total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL). Activity of liver marker enzymes such as SGOT and SGPT were also determined.

**Estimation of lipid peroxides (LPO)**

The level of LPO in plasma was determined in terms of thiobarbituric acid-reacting substances (TBARS) by the method of Draper and Hardley. The value was expressed as nM/mL plasma.

**Estimation of glutathione and antioxidant enzymes**

Plasma glutathione (GSH) and serum enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined to assess the antioxidant status in rats received HFD with/without HAEPA.
lipase activity was estimated by the method of Korn et al. Equal volumes of ice cold 5% TCA was added to the plasma and the precipitated proteins were removed by centrifugation. The supernatant was used for determination of GSH by using DTNB. GPx (EC: 1.11.1.9) was assayed by the method of Flohe and Gunzler. The GPx activity was expressed as U/mL. SOD (EC: 1.15.1.1) activity was measured according to the method of Kakkar et al. Inhibiting the formation of nitroblue-tetrazolium to blue coloured formazone in the presence of phenazine methosulfate and NADH was measured at 560 nm using n-butanol as blank. The enzyme activity was expressed as U/dL. Decomposition of H$_2$O$_2$ in the presence of CAT (EC: 1.11.1.6) was kinetically measured at 240 nm. CAT activity was defined as the amount of enzyme required to decompose 1 µM of H$_2$O$_2$/min. The enzyme activity was expressed as U/mL.

Assay of lipid metabolic key enzymes

The activity of HMG-CoA reductase was assayed indirectly by assessing the ratio of HMG-CoA to mevalonate in liver as described by Philipp and Shapiro. The FAS activity in liver was determined by measuring malonyl CoA dependent oxidation of NADPH at 37°C. One unit of enzyme activity represents 1 mM of NADPH oxidized per min at 37°C. Protein concentration was determined by using bovine serum albumin as standard. Lipoprotein lipase activity was estimated by the method of Korn with modifications. Values were expressed as µmoles of glycerol liberated/h/g tissue.

Histopathological evaluation

Liver and adipose tissue samples were fixed in 10% neutral buffered formalin for 24 h. Ultra-thin sections of the tissues were cut from embedded tissue blocks. The sections were then stained with hematoxylin-eosin and observed under light microscope.

Statistical analysis

Data were analysed by using commercially available statistics software package (SPSS for window V. 10). The statistically significant variation between different groups was determined by applying one way ANOVA with post hoc Bonferroni test and the P value <0.05 was considered significant.

Results

Effect of HAEPA on weight gain, BMI and total fat pad mass (TFP)

The weight gain, BMI and TFP of control and experimental rats are shown in Table 1. A significant increase in weight gain, BMI and TFP (p=0.000) were observed in HFD fed rats (Gr. III) when compared to control rats (Gr. I). HAEPA co-administered rats showed significant reduction in body wt. gain, BMI and TFP. The effect was more significant in Gr. VI rats which received 100 mg/kg body wt. of HAEPA.

Effect of HAEPA on blood lipids

Figure 1 shows the plasma levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). There was a significant (p=0.000) increase in TC, TG, LDL and significant (p=0.000) decrease in the HDL levels in HFD fed rats than in normal diet fed rats. HAEPA co-administration increased the HDL level significantly (p=0.000) and decreased the levels of TC, TG and LDL in HFD fed rats.

Table 1—Effect of various concentration of HAEPA on weight gain, BMI and total fat pad mass (TFP) in control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Net weight gain (g) (Final-Initial)</th>
<th>BMI (g/cm$^2$)</th>
<th>TFP mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr. I (Control)</td>
<td>143.9±14.53</td>
<td>0.59±0.07</td>
<td>14.7±1.60</td>
</tr>
<tr>
<td>Gr. II (HAEPA Control)</td>
<td>139.4±16.17NS</td>
<td>0.61±0.09NS</td>
<td>12.3±1.77NS</td>
</tr>
<tr>
<td>Gr. III (HFD)</td>
<td>274±30.69*</td>
<td>1.54±0.19*</td>
<td>23.4±3.44*</td>
</tr>
<tr>
<td>Gr. IV (HFD+HAEPA)</td>
<td>193.5±25.73*</td>
<td>0.86±0.12*</td>
<td>16.9±2.21*</td>
</tr>
<tr>
<td>(25 mg/kg body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr. V (HFD+HAEPA)</td>
<td>168.4±24.25*</td>
<td>0.79±0.11*</td>
<td>15.6±2.0*</td>
</tr>
<tr>
<td>(50 mg/kg body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr. VI (HFD+HAEPA)</td>
<td>158.2±19.93*</td>
<td>0.69±0.08*</td>
<td>12.8±1.63*</td>
</tr>
<tr>
<td>(100 mg/kg body wt.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr. VII (HFD+HAEPA)</td>
<td>160.4±20.96*</td>
<td>0.67±0.07*</td>
<td>11.8±1.19*</td>
</tr>
<tr>
<td>(200 mg/kg body wt.)</td>
<td></td>
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</tr>
</tbody>
</table>

[Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HAEPA control, Control vs. HFD, HFD vs. HFD + HAEPA. *P=0.000, NS=Non significant]
Effect of HAEPA on LPO and antioxidants in plasma

The plasma levels of LPO, GSH and serum SOD, CAT and GPx activity of control and experimental rats represented in Table 2. HFD control rats showed low level of reduced glutathione and enzymatic antioxidants when compared to normal control rats. An improved antioxidant status was observed in HAEPA co-administered rats in a dose dependent manner. LPO level was found to be significantly decreased \((p=0.000)\) in HAEPA co-administered rats at the concentration of 100 and 200 mg/kg body wt.

Effect of HAEPA on the level of liver marker enzymes and total lipid

We observed a significant increase \((p=0.000)\) in the activity of SGOT, SGPT and the level of total lipid content in the liver of HFD control rats than in normal control rats (Fig. 2). HAEPA co-administered rats

![Fig. 1—Effect of various concentration of HAEPA on total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) in the plasma of control and experimental rats. [Values as mean ± SD for 6 animals in each group. Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HAEPA control, Control vs. HFD, HFD vs. HFD + HAEPA. *\(p=0.000\); NS=Non significant]![Fig. 2—Effect of various concentration of HAEPA on liver marker enzymes and total lipid of control and experimental rats. [Values as mean ± SD for 6 animals in each group. Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HAEPA control, Control vs. HFD, HFD vs. HFD + HAEPA. *\(p=0.000\); NS=Non significant]![Table 2—Effect of various concentration of HAEPA on plasma LPO, GSH and serum enzymatic antioxidant levels of control and experimental rats [Values as mean±SD for 6 animals in each group] Groups LPO (nM/mL) GSH (mg/dL) SOD (U/dL) CAT (U/mL) GPx (U/mL) Gr. I (Control) 0.20±0.02 26.32±3.08 18.36±2.68 6.75±0.94 16.14±2.32 Gr. II (HAEPA Control) 0.19±0.02 \(^{NS}\) 27.9±3.4 \(^{NS}\) 18.61±2.69 \(^{NS}\) 7.12±0.85 \(^{NS}\) 15.97±2.28 \(^{NS}\) Gr. III (HFD) 0.52±0.05 * 15.21±1.96 * 11.03±1.18 * 4.32±0.64 * 8.46±1.15 * Gr. IV (HFD+HAEPA (25 mg/kg body wt.) 0.41±0.05 * 19.87±2.16 \(^{δ}\) 14.12±1.72 \(^{δ}\) 5.64±0.72 \(^{δ}\) 11.23±1.39 \(^{δ}\) Gr. V (HFD+HAEPA (50 mg/kg body wt.) 0.35±0.04 * 21.17±3.13 \(^{a}\) 15.42±1.63 \(^{a}\) 6.12±0.62 * 13.14±1.37 * Gr. VI (HFD+HAEPA (100 mg/kg body wt.) 0.25±0.03 * 23.67±2.91 * 16.91±1.84 * 7.23±0.82 * 15.83±1.77 * Gr. VII (HFD+HAEPA (200 mg/kg body wt.) 0.23±0.03 * 24.62±2.78 * 16.23±1.80 * 6.89±0.85 * 16.12±1.72 * [Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HAEPA control, Control vs. HFD, HFD vs. HFD + HAEPA. *\(p=0.000\), \(^{δ}\)\(p=0.016\), \(^{a}\)\(p=0.001\), \(^{b}\)\(p=0.008\), \(^{l}\)\(p=0.037\), NS=Non significant]
showed significant decrease in the levels of SGOT, SGPT and total lipid in a dose dependent manner up to the dose of 200 mg/kg body wt.

Effect of HAEPA on key enzymes of lipid metabolism

Table 3 represents the activities of fatty acid synthase (FAS), lipoprotein lipase and HMG CoA reductase in the liver of control and experimental rats. We observed a significant increase (p=0.000) in the activity of HMG CoA reductase, FAS and a significant decrease (p=0.000) in the activity of lipoprotein lipase in HFD fed rats compared to normal diet fed rats. HAEPA co-administered rats showed significant (p=0.000) decrease in the activities of HMG CoA reductase and FAS. Lipoprotein lipase activity in HAEPA+HFD rats showed significant (p=0.000) increase.

Histopathology of liver and adipose tissue

Photomicrograph of liver isolated from HFD fed rats (Gr. III) showed cell necrosis, microvesicular steatosis and accumulation of fat droplets (Fig. 3C). These changes significantly reduced in the liver of HAEPA (100 and 200 mg/kg body wt.) co-administered rats (Fig. 3F & G). Photomicrograph of visceral adipose tissue showed hypertrophied adipocytes in HFD fed rats (Gr. III). Many regions of adipocytes with normal cell size were observed in HAEPA co-administered rats (Fig. 4F & G).

Discussion

Hyperlipidemia and obesity are the major risk factors for type 2 diabetes mellitus and cardiovascular diseases. Eating high-fat diet increases atherogenic index and undermine glucose metabolism in skeletal muscle which is the major site of insulin-stimulated glucose disposal. Motshakeri et al stated that high sugar high fat diet possibly increased the insulin resistance in Sprague Dawley rats. Novelli et al. also reported that high caloric diet significantly increased the BMI of HFD fed rats compared to the rats fed standard diet. In the present study, avocado fruit extract was found to decrease the atherogenic effect of HFD significantly at the dosage of 100 mg/kg body wt.
The HFD used in the present study significantly increased the plasma TC, TG and LDL levels. HAEPa has showed promising lipid lowering effect especially on LDL cholesterol. Avocado fruit is a rich source of beta-sitosterol, an anti-cholesterolemic agent and has been shown to reduce blood lipid levels probably by inhibiting the intestinal absorption of cholesterol. Further, hypolipidemic drugs with antioxidant properties are known to prevent LDL peroxidation and its elevation in blood. The LDL lowering and the HDL increasing effect of HAEPa might be due to the presence of natural antioxidants, vitamin C, E and carotenones present in the avocado extract which inhibit LDL peroxidation. Cholesterol transport to extra hepatic tissues is by LDL while HDL has an important role in reversing the cholesterol transport. Therefore, HDL exerts a protective effect against coronary heart disease induced by hypercholesterolemia.

The increased plasma cholesterol level in HFD fed rats could be due to enhancement of de novo synthesis in liver. Nabel reported that the elevated plasma LDL level is attributed to impaired activity of hepatic LDL receptors, which normally clear LDL from the plasma.

Concerning the plasma level of triglycerides (TG), the present finding is in agreement with Supkamonseni et al. who had also demonstrated that plasma TG level increased significantly after feeding rats with HFD which was later suppressed by Centella asiatica (L.) extract. Earlier, Kritchevsky et al. observed that the TG composition, structure as well as chain length of fatty acids in dietary fat are important determinants of atherogenicity. We too, have observed in this study that avocado fruit extract reduced the level of TG significantly in HFD+HAEPa rats.

Fatty acid synthase (FAS) is a multienzyme complex which plays a key role in fatty acid biosynthesis. Here, we observed a significant increase in FAS activity in HFD fed rats than in those fed with HFD+HAEPa. HMG CoA reductase catalyses the conversion of HMG CoA to mevalonate. The lower ratio points to higher enzyme activity and vice versa. The significant increase in HMG CoA:mevalonate ratio as observed in HFD rats indicates higher HMG CoA reductase activity in HFD control rats than in those fed with HFD+HAEPa. These results are further supported by high level of blood cholesterol in HFD control rat group. The inhibition of HMG CoA reductase by HAEPa is well evidenced in this study. Also, we found significant decrease in the activity of lipoprotein lipase (LPL) in HFD control rats. LPL plays a vital role in the catabolism of triacylglycerol and release free fatty acids from lipoproteins. In HAEPa co-administered rats the enzyme activity is increased significantly than in HFD only fed rats.

High level intake of saturated fat leads to increased fat mediated oxidative stress and decreased antioxidant status. Oxidative stress induced by free radicals is the leading cause of several diseases such as cancer, ulcer, rheumatoid arthritis, cardiovascular, reproductive and neurodegenerative diseases. Oxidation of the lipid core of low density lipoproteins leads to a change in the lipoprotein conformation. The oxidized LDL recognised by monocyte/macrophage of the arterial wall and develops into atherosclerotic plaques. The fat substituted in HFD in the present study was of saturated in nature and this could have led to free radicals formation in excess.

Increased caloric intake is an important factor in decreasing the mitochondrial membrane fluidity and increasing the generation of ROS and reactive nitrogen species. Under normal conditions,
antioxidant enzyme superoxide dismutase catalyse the conversion of superoxide radicals \( \text{O}_2^- \) into hydrogen peroxide \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) and catalase and glutathione peroxidase further detoxifies \( \text{H}_2\text{O}_2 \).

Glutathione, a tripeptide thiol found in all cells, in metabolism, transport and cellular protection. It takes part in reducing disulfides and other molecules, and conjugate with compounds of exogenous and endogenous origin. Glutathione remove toxic intermediates of free radicals by reducing hydroperoxides in the presence of GPx. Glutathione also functions as a free radical scavenger and in the repair of free radical induced damage in biomolecules. The decrease in GSH level observed in HFD fed rats might represents increased utilization due to oxidative stress. HAEPA significantly prevented the depletion of GSH in HFD fed rats probably by its antioxidant components.

The enzymes SOD, CAT and GPx are the first line of cellular defence against oxidative injury and act to dispose superoxide anions and hydrogen peroxide. The decreased activities of these enzymes in the present study is supported by the findings of Durkar et al. who stated that hyperlipidemia is associated with decreased antioxidant status. Das et al. have shown that the lipid lowering effect of \textit{Moringa oleifera} seed oil is attributed to the natural antioxidants present in the oil which exhibit free radical scavenging activities. HAEPA co-administration restored the levels of SOD, CAT, GPx and GSH in HFD fed rats probably due to the antioxidants rich nature of avocado fruit extract.

In the present study, the liver damage induced by HFD promoted the serum transaminases and fat mass in liver. The alleviating effect of HAEPA on HFD induced changes in liver is well supported by the histopathological observations.

**Conclusion**

The present study has demonstrated that the hydroalcoholic fruit extract of \textit{Persea americana} (HAEPA) is a potent hypolipidemic agent probably by reducing the activities of HMG CoA reductase and FAS, the key metabolic enzymes of liver which regulate lipid homeostasis. Among the four doses studied, 100 mg/kg body wt. exhibited optimum lipid lowering effect against HFD induced obesity.

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**References**


