A Comparative Study on the Effects of Ethanolic and Aqueous Extracts of *Psidium guajava* Fruits in Hyperlipidemic Rats

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Introduction

Hyperlipidemia is a clinical condition characterized by the elevated levels of lipid parameters and is one of the major risk factors for coronary heart diseases. Elevated level of total cholesterol (TC), low density lipoproteins cholesterol (LDL-C) and triglycerides (TG) in blood are recognised as major risk factors for coronary heart diseases. Other complications related to hyperlipidemia are artherosclerosis, hypertension and obesity (NECP 2002).

Globally, a third of ischaemic heart disease is attributable to high cholesterol levels. According to World Health Statistics 2011, overall raised cholesterol levels have been estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYS), or 2.0% of total DALYS (Roth *et al.,* 2011).

Elevated lipid levels result from increased absorption through the gut or through enhanced endogenous synthesis. Hence, hyper-lipidemia can be reduced by two possible ways, viz., by blocking endogenous synthesis or by decreasing absorption. Both these factors can be evaluated in normal animals without artificial diets using Triton WR 1339 (Moss *et al.,* 1971).

Currently available hypolipidemic drugs include statins and fibrates. The former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the later acts by enhancing the clearance of TG rich lipoproteins (Mahley *et al.,* 2006). However, consumption of these synthetic drugs have been associated with side effects such as hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Kumar *et al.,* 2008). Hence, there is an increased demand for newer herbal drugs with an ability to reduce or regulate serum TC and TG concentrations. As herbal medicines are less damaging than synthetic drugs they have better patient tolerence even on long term use (Kaliora *et al.,* 2006).

The present study is aimed at evaluating the potential of ethanolic and aqueous extracts of *Psidium guava* (PG) fruits, which are cheaper, in reducing the lipid parameters.

Materials and Methods

Collection of plant material

The *Psidium guajava* (guava) fruits were collected from Trichy local market. The fruits were dried at room temperature and then reduced to coarse powder.

This powder (50 g) was extracted with ethanol (300 ml) in soxhlet apparatus at 80°C. Aqueous solution of dried extracts was also prepared in the same manner and used for pharmacological testing.

Preparation of 2% cholesterol diet

2 g of cholesterol (extra pure, Scharlau spain) and 500 mg of Cholic acid (min 98%, sigma aldrich) was thoroughly mixed and mashed with 97.5 g of rat pellet diet. The mixture was made into a pellet form (Rabiea Bilal *et al.*, 2011) used as feed for the experimental animals.

Experimental animals

Male albino rats (Wistar strain) weighing between 110-150 gm were maintained at 25 to 30°C and kept in well ventilated animal house under natural photoperiodic condition in large polypropylene cages and were fed standard rats chow and water *ad libitum*. After complete acclimatization to the lab condition, a total of 30 healthy albino rats were selected and, randomly divided into five groups. Each group had six animals.

Experimental design

The Group I animals served as control and had free access to food and water for 21 days. The animals in Group II served as experimental and were provided with cholesterol rich diet and water for 21 days. The animals in group III were treated with Lovastatin at a dose of 20 mg/kg body weight and coadministered with the cholesterol rich diet and water for 21 days.

The animals in group IV were treated with ethanolic extract of *Psidium gaujava* fruits 200 mg/kg body weight daily, along with the cholesterol diet and water for 21 days. Group V animals were treated with aqueous extract of *Psidium guajava* fruits (200 mg/kg body weight) daily along with the high cholesterol diet (HCD) and water for 21 days. After the experimental period was over, the rats were sacrificed by cervical decapitation.

The blood samples were collected aseptically and stored in a sterile container. Serum samples were prepared and utilized for biochemical estimations. The tissues were removed surgically and subjected to histological studies using standard procedures.

The biochemical parameters studied were:

1. Estimation of serum cholesterol (Zak *et al.*, 1954)
2. Estimation of serum HDL cholesterol (Burnstein *et al.*, 1970)
3. Estimation of LDL cholesterol (calculation)
4. Estimation of triglycerides (Butler *et al.*, 1961)
5. Estimation of phospholipids (Fisk and Subbarow *et al.*,1925)

Statistical analysis

The data obtained from the biochemical estimations were subjected to student's t test. Test values of $p < 0.05$ were considered as statistically significant. Data were presented as mean ± standard deviation.

Results and Discussion

Table-1: Serum Cholesterol Level

Groups	Serum Cholesterol (mg/dl)
Group-I (Normal Control)	44.635±4.058
Group- II (Hyperlipidemic Control)	113.33±6.360
Group-III (Lovastatin standard +HCD)	79.995±4.047
Group-IV (PG ethanol extract + HCD)	44.635±1.937
Group-V (PG aqueous extract +HCD)	68.42±6.720

(Values are expressed as Mean ±SD)
Group I vs Group II:*Significant at $p<0.05$
Group I vs Group IV:* Non Significant at $p<0.05$

Table 1 represents the levels of serum cholesterol in different experimental animal groups. A significant increase in ($p < 0.05$) serum cholesterol level was observed in animals fed with high cholesterol diet, when compared to normal group. In Group IV animals, treatment with a *Psidium guajava* ethanol extract balanced the serum cholesterol level and as a result a decrease in serum cholesterol was observed. Group III animals treated with standard drug (Lovastatin) also shows a level that is closer to the normal cholesterol level. Group V animals also show normal serum cholesterol level, when compared to that of group II.

From the results, it is known that the difference between the levels of cholesterol in normal and ethanolic extract treated animals is not statistically significant. So the ethanolic extract of *Psidium guajava* is powerful in maintaining the blood cholesterol level.

The level of cholesterol in group V animals is lower than that of animals in hyperlipidemic control group. So, ethanolic extract is more powerful in bring the elevated level of cholesterol than the aqueous extract.

Increase in cholesterol level in serum because of high fat intake has already been registered in literature. Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increase free fatty acid flux to the liver (e.g. after the fatty meal or in the situation of increased lipolysis) but due to anti-hyoerlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte

cholesterol concentration by increase the catabolic conversation of cholesterol to bile acids in liver. High cholesterol diet increased serum cholesterol and LDL-C level significant. A rise in LDL may cause deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease (Dipa *et al*., 2010).

Table-2: Serum Triglycerides Level

Groups	Serum Triglycerides (mg/dl)
Group-I (Normal Control)	58.75±2.331
Group- II (Hyperlipidemic Control)	78.75±1.831
Group-III (Lovastatin standard +HCD)	61±1.830
Group-IV (PG ethanol extract + HCD)	60±2.951
Group-V (PG aqueous extract +HCD)	59.75±2.692

(Values are expressed as Mean ±SD)
Group I vs Group II :*Significant at $p<0.05$
Group I vs Group IV, V :* Non Significant at $p<0.05$

Table 2, represents the levels of serum triglycerides in different experimental animal groups. A significantly increase in serum triglycerides was observed in the animals of hyperlipidemic control group ($p < 0.05$), when compared to normal group. In the animals of Group IV ,treatment with *Psidium guajava* ethanol extract reduced serum triglyceride level ,when compared to that of group II. In the animals of Group III treated with standard drug, the level of triglycerides is maintained near normal. Animals of group V have triglyceride levels closer to normal (the difference is statistically not significant; $p= 0.5$) because of the effects of aqueous extract of *Psidium guajava*.

The level of triglycerides is brought back to near normal by the action of extracts of *Psidium guajava* at a dose of 200mg/kg body weight per day. Similar effects of plants have been found in literature. In an analysis aimed at evaluating the antihyperlipidemic activity, it was found that the ethanolic extract of *Rhinacanthus nasutus* reduced elevated levels of triglycerides in hyperlipidemic rat models (Brahma *et al* , 2013).

Table 3 represents the levels of serum LDL in different experimental animal groups. A significant increase in serum LDL was observed in animals of hyperlipidemic group, when compared to normal group ($p < 0.05$). In animals of Group IV, treatment with a *Psidium guajava e*thanol extract reduced serum LDL level. As a result, significant decrease in serum LDL was registered in group IV, when compared to that of group II. This reduction could be due to the action of the phytochemicals present in the ethanolic extract of *Psidium guava* fruits.

Table-3: Serum Low Density Lipoproteins (LDL) Level

Groups	Serum LDL Level (mg/dl)
Group-I (Normal Control)	49.817±2.787
Group- II (Hyperlipidemic Control)	76.26±2.745
Group-III (Lovastatin standard +HCD)	50.21±6.354
Group-IV (PG ethanol extract + HCD)	47.31±2.951
Group-V (PG aqueous extract +HCD)	48.345±2.692

(Values are expressed as Mean ±SD)
Group I vs Group II :*Significant at $p<0.05$
Group I vs Group IV, V :* Non Significant at $p<0.05$

In the animals of Group III treated with standard drug (Lovastatin), the level of LDL is maintained closer to normal and the difference is statistically not significant. The reduction in the level of LDL in Lovastatin treated animals is due to the inhibition of HMG CoA reductase involved in cholesterol biosynthesis (Istvan *et al.*, 2001).

Reduction in the elevated levels of LDL in experimental animals like mice and rats have been registered already in the literature. Pooja *et al*. (2009) when carried out an investigation to determine the antihyperlipidemic potential of *HIbiscus sabtariffa,* it was found to reduce the level of LDL in hyperlipidemic rats. The observation of this present study also follows the same.

Table-4: Serum High Density Lipoproteins (LDL) Level

Groups	Serum HDL Level (mg/dl)
Group-I (Normal Control)	85.52±3.787
Group- II (Hyperlipidemic Control)	67.1±4.745
Group-III (Lovastatin standard +HCD)	78±6.354
Group-IV (PG ethanol extract + HCD)	84.2±2.951
Group-V (PG aqueous extract +HCD)	80.23±2.692

(Values are expressed as Mean ±SD)
Group I vs Group II :*Significant at $p<0.05$
Group I vs Group IV:* Non Significant at $p<0.05$

Table-4 represents the levels of serum HDL-cholesterol levels in different groups. A significant decrease in serum HDL-cholesterol was observed in Group II (induction) when compared to level in the animals of group I. A significant increase in serum HDL-cholesterol was observed in the rats of

group IV treated with the ethanolic extract of *Psidium guajava* when compared to that of group II. The rats in group V have significantly increased the level of HDL when compared to levels in the rats of group II.

The HDL cholesterol is the transport form of cholesterol from peripheral tissues to liver where they are excreted as bile acids. The ethanolic and aqueous extracts of *Psidium guajava* could promote the level of HDL through the activity of the phytochemicals present in them.

There are many fruits with curative properties towards reducing obesity. In effort to analyse the antihyperlipidemic activity of *Helicteres isora*fruit extract in Diabetes mellitus, Boopathy *et al.* (2010) induced hyperglycemia in rats by infusing streptozotocin. Later the team found that the extract was capable of increasing the HDL cholesterol level. The findings of the present study also coincides with the result of the above.

Table-5: Serum Phospholipids Level

Groups	Serum Phospholipids Level (mg/dl)
Group-I (Normal Control)	97.33±3.787
Group- II (Hyperlipidemic Control)	118.1±8.946
Group-III (Lovastatin standard +HCD)	92.96±5.354
Group-IV (PG ethanol extract + HCD)	95.25±2.951
Group-V (PG aqueous extract +HCD)	94.6±2.692

(Values are expressed as Mean ±SD)
Group I vs Group II : Significant at $p<0.05$
Group II vs Group III, V: Non Significant at $p<0.05$
Group II vs Group IV: Significant at $p<0.05$

In lipid profile, the serum phospholipids were significantly increased ($p < 0.05$) in animals belonging to Group II when compare to Group I, III, IV and V (Table-2). The concentration of phospholipids increased during the hypercholesterolemia in the animals fed with high cholesterol diet (HDC). Decrease in the phospholipids level in Group IV (HCD+ethanolic extract of *Psidium guajava*) and V (HCD+aqueous extract of *Psidium guajava*) its shows that the extract *of Psidium guajava* is capable of reducing level of phospholipids.

Group-II animals receiving cholesterol showed a significant increase in phospholipids levels when compared to that of the normal group (G-I). Rats treated with standard drug (G-III) had significantly lowered phospholipids level when compared to the cholesterol treated group (G-II).

The reduction in the levels of phospholipids in the animals of group IV could be due to the action of the phytochemicals presents in the ethanolic extract of *Psidium guajava*. The reduction in the levels of phospholipids , by treatment with plant extracts have been documented in the literature.

Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes, and death. Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increase free fatty acid flux to the liver (e.g. after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increase in the catabolic conversation of cholesterol to bile acids in liver. High cholesterol diet increased serum cholesterol and LDL-C level significant. A rise in LDL may cause deposition of cholesterol in arteries and aorta and hence it is a direct risk factor for coronary heart disease. A significant decrease in serum phospholipids was observed in animals treated with *Terminalia chebula* extract at 200 mg/kg dose (G-V) (Dipa *et al*., 2010).

From the above analysis, it is also obvious that the efficacy of both the extracts in reducing the elevated levels of lipid parameters is nearly the same.

Histopathology

In the histopathological study, high cholesterol diet fed rats shows fatty Cytoplasmic vaculated cellsascompared to normal control. Treatment with 200 mg extract of *Psidium guaajva* shows less fatty cytoplasmic vacuoles as compared to high cholesterol diet fed rats. In histopathological study, we found that treatment with *Psidium guajava* significantly decreases the plaque size in aorta and significantly decrease fatty cytoplasmic vacuaoated cells in Liver parenchyma and liver cell necrosis is prevented.

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