A Comparative Study on the Effects of Ethanolic and Aqueous Extracts of *Premna serratifolia* leaves in Hyperlipidemic Male Albino Rats

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Introduction

Lipids are one of the major constituents of food and provide the major source of energy. This group of substances includes, triglycerides (TG), diglycerides, monoglycerides, free fatty acids, phospholipids, and cholesterol, but the triglyerides are the major component of most food. This represents ninety five to ninety nine percentage of the total lipids present in the body. The most important precursor of derived lipids is the cholesterol, the best known sterol, and it is a precursor of a large number of steroid hormones. Cholesterol is present mainly in blood in the lipoprotein fractions as low density lipoprotein (LDL) and high density lipoprotein (HDL) (Wierzbicki *et al*., 2005).

Hyperlipidemia is an excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood, attached to proteins. This is the only way that these fatty substances canremain dissolved while in circulation.

It is a highly predictive risk factor for atherosclerosis, coronary artery disease and cerebral vascular disease. Atherosclerosis of arteries is a generalized disease of the arterial network known as a progressive and silent killer disease characterized by the formation of large and or medium sized coronary arteries and which reduces blood flow to the myocardium called coronary artery diseases. Hyperlipidema and hypercholesterolemia are not only secondary metabolic dysregulation associated with diabetes but also represent increased risk factors for development of diabetes. Several factors, such as, life style, as diet rich in cholesterol, age and hypertension, have been reported to cause heart failure (Schaefer *et al*.,1995).

High levels of cholesterol, particularly low density lipoprotein cholesterol are mainly responsible for hypercholesterolemia. Recently, it has been found that hypercholersterolemiais also associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of low density lipoprotein is a major factor in the vascular damage associated with high cholesterol levels. Hence the inhibition of hypercholesterolemia is considered to be an important therapeutic approach and efforts have been made to identify the antihyperlipidemic effects of various medicinal plants (Hu *et al*., 2006).

Medications most commonly used to treat high LDL levels are statins, such as atorvastatin or simvastatin. These medications work by reducing the production

of cholesterol within the body. Although safe and effective, statins cause muscle damage, typically when used in combination with other medications.

As a result, phytotheraphy has taken up new dimensions in its approachtowards the betterment (progress) of mankind in the area of medicinal plants which area rich source of vitamins, minerals, specialized substances that are of great value. This study aims to analyze the effects of ethanolic and aqueous extracts of *Premna serratifolia* in hyperlipidemicmale albino rats.

Materials and Methods

Collection of plant material

Plants were collected from Kolli hills, Nammakal district in the month of January 2012 .The plant was identified and authenticated by the taxonomist John Britto, Director, Rapinat Herbarium, St.Joseph's College, Trichy .

Preparation of Plant Extract

Leaves of *Premna serratifolia* were shade dried for a period of three weeks and then the dried leaves were powdered and used for extraction. 50 go f dried powder of *Premna serratifolia* leaves were taken in a Soxhlet apparatus and soaked in 300 ml of ethanol, and water separately. The separation process was carried out till complete extraction was achieved.

Preparation of 2% cholesterol diet

2 g of cholesterol (extra pure, Scharlauspain) 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).

Experimental animals

Male albino rats weighing 150-200 gm were used for experiment. These animals were reared, providing rodent pellet diet and water in the animal house, which was well ventilated and lighted. A total of healthy 30 albino rats were selected and acclimatizedto the lab conditions for 15 days and then randomly divided into five groups of six each.

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 animalsin groupIII were treated with Simvostatin at a dose of 10 mg/kg body weight along with the cholesterol rich diet and water for 21 days.The animals in group IV were treated with ethanolic extract of *Premna serratifolia* leaves 200 mg/kg body weight daily, along with the cholesterol diet and water for 21 days.Group Vanimals were treated with aqueous extract of *Premna serratifolia* leaves (200 mg/kg body weightorally) daily along with the cholesteroldiet and water for 21 days. After the experimental period wasover, 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's *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

The levels of various biochemical parameters are depicted in the following table.

Serum cholesterol level

The serum cholesterollevel has significantly increased in cholesterol treated group (G-II) when compared with the normal group (G-I) ($p < 0.001$). A significant decrease in the levels of serum cholesterol was observed on administration of Simvastain (G-III), when compared with the cholesterol treated group (G-II) (Table- 1).

Table 1

Groups	Serum cholesterol (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)
Group I (Normal)	116.6±14	45.31±8.2	82.25±2.89
Group II (High cholesterol)	171.3±8	23.64±2.6	137.92±1.96
Group III (Simvostatin + High cholesterol)	112.5±15	47.33±2.7	86.11±1.52
Group IV (Ethanol extract+ High cholesterol)	125±14	43.46±5	85.31±1.30
Group V Aqueous extract + High cholesterol)	114.5±12	39.92±4.5	81.40±1.56

Table 2

Groups	Serum TGL (mg/dl)	Serum Phospholipids (mg/dl)
Group I (Normal)	66.25±2.33	51.87±1.8
Group II (High cholesterol)	100±4.4	96.25±5.33
Group III (Simvostatin + High cholesterol)	68.75±1.72	52.20±3.24
Group IV- (Ethanol extract+ High cholesterol)	71.25±5.9	53.12±3.72
Group V Aqueous extract + High cholesterol)	67.5±1.83	59.79±2.28

The level of cholesterol in group IV animals (High cholesterol + ethanolic extract treated) is brought back to near normal. The difference between the levels of cholesterol of the two groups namely group I and Group IV is not statistically significant (p=0.32).

The reduction in the cholesterol level during the treatment with lovastatin and plant extract have been documented in the literature. In an experiment, conducted to study the antihyperlipidemic effect, the *Tageteserecta* extract at 200 mg/kg (G-V) caused a significant decrease in the serum cholesterol when compared to the cholesterol treated group (G-II) (Rodda Raghuveer *et al*., 2011). The result of the present study also coincide with the observation of the above work.

Serum HDL level

The serum HDL cholesterol was significantly decreased in animals belonging to Group II when compared to those of Group I, III, IV and group V(Table -1). The animals of Group III, IV and Group V had significantly increased serum HDL cholesterol levels than Group II. The concentration of HDL cholesterol decreased during hyperlipidemia. The increase HDL cholesterol in Group III, IV and Group V than Group II shows that the extract of *Premna serratifolia* is capable of increasing HDL cholesterol levels thereby decreasing the levels of bad cholesterol (LDL) and exhibits hypolipidemic effect.

The rats fed with high cholesteroldiet (G-II) showed a significant decrease in HDL levels when compared to the normal group (G-I). Group-III, receiving standard drug Simvastatin showed a significant increase in HDL levels when compared to the control group (G-II).

HDL cholesterol is the form in which cholesterol is transported back to liver from peripheral tissues for excretion. Reduction the HDL level represents

accumulation of cholesterol in the periphery. During elevated levels of LDL, reduction in the HDL level have been recorded in the previous studies.

In an investigation to find the cholesterol lowering effect of fruits, animals were fed with high cholesterol diet (HCD). High cholesterol diet significantly increased the level of liver TC-LDL-C and TG with less concentration of HDL-C compared to baseline. There was no statistically significant difference in liver lipid profile between groups at baseline. After 8 weeks of treatment with *Luffa aegyptiaca* fruits the concentrations of TC, LDL-C and TG were significant lower, with an increased HDL-C concentration in treatment group as compared to control group (Abdul *et al*., 2011)

Serum LDL level

The Low density Lipoprotein were significantly increased in animals belonging to Group II when compared to that of Group I, III, IV, V (Table-1). The concentration of LDL increased during the hypercholesterolemia in the animals fed with high cholesterol diet. Decrease in the LDL level in Group IV (HCD+ ethanolic extract of *Premna serratifolia* leaves) and V (HCD + aqueous extrac*t of Premna serratifolia* leaves) could be due to the ability of the phytochemicals present in the extract of *Premna serratifolia*.

Similar results have been found in the literature. The rats induced with cholesterol (G-II) a significant increase in LDL levels was observed when compared to the normal group (G-I). Group-III animals, receiving standard drug showed a significant decrease in LDL levels when compared to the control group (G-II). Administration of *Tageteserecta*extract at dose of 200 mg/kg (G-V) has shown a significant decrease in LDL levels (Rodda Raghuveer *et al*., 2011).

In another clinical analysis, a rat fed with high cholesterol for 7 days exhibited significant increase in TC,TG,LDL-C and VLDL and significant decrease in HDL-C, HDL-C, HDL-C ratio as compared to the normal animals. Treatment with atorvastatin (10 mg kg b.wt., p.o.) showed significant decrease in elevated TC,TG,LDL-C and VLDL, with significant increase in HDL-C ($p < 0.05$) as compared to the high cholesterol diet control. Whereas treatment with hydroalcoholic extract of *Gymnema* leaves at a dose of 200 mg\ kg\b.wt.,p.o showed significant decrease in the elevated levels of TC,TG,LDL-C and VLDL, with significant increase in the HDL-C ($p < 0.05$) as compared to the high cholesterol diet control (Rachh *et al*., 2010).

Serum triglyceride (TGL) levels

The serum triglycerides were significantly increased in animals belonging to Group II when compared to that of Group I, III, IV and V (Table-2).The difference is statistically significant at 95% confidence level ($p < 0.001$) The concentration of triglycerides increased during the hyperlipidemia in the animals fed with high cholesterol diet. Decrease in the triglycerides level in Group IV (HCD + ethanolic extract of *Premna serratifolia* leaves) and V (HCD

+ aqueous extract of*Premna serratifolia* leaves) could be due to the antihyperlipidemic effect of *Premna serratifolia* leaves.

There is no statistically significant difference existing between the levels of triglycerides of group III and group IV animals (p=0.259). So, it is understood that the efficacy of ethanolic extract of *Premna serratifolia* is comparable to that of simvastatin.

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

Similar observations have been documented already in the literature. The oral administration of high fat diet for 28days to rats produced a significant ($p < 0.01$) increase in serum TC, LDL-C, VLDL-C and triglycerides as compared to normal control rats. These significant rises were accompanied by significant ($p < 0.01$) decline of serum HDL-C as compared to normal control rats. The treatment with HC (200 mg/kg) and standard drug atorvastatin (10 mg/kg/day p.o.) to high fat rats resulted in significant ($p < 0.01$) decline in serum TC, LDL-C, VLDL-C and triglycerides as compared to hyperlipidemic control rats. Further, atorvastatin treated group significantly increased the serum HDL-C level in high fat induced rats Whereas, treatment with HC extract did not modulate the reduced serum HDL-C level (Shivali *et al*., 2010).

In another work of the same kind, A significant decrease in serum triglycerides was observed in animals treated with *Hibiscus sabdariffa* Linn extract at 200 mg/kg dose (G-V) (Pooja *et al*., 2009).

Serum phospholipid levels

In lipid profile, the serum phospholipids were significantly increased 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. Decrease in the phospholipids level in Group IV (HCD+ ethanolic extract of *Premna serratifolia* leaves) and V (HCD + aqueous extract of *Premna serratifolia* leaves) its shows that the extract of *Premna serratifolia* 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 *Premna serratifolia*. 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

The liver of group I shows regular pattern of arrangement of cells whereas the liver of group II animals fed with high cholesterol shows increased vacuolation. The liver of simvastatin treated animals in group III is not affected to a greater extent. The architecture of liver tissue in animals of group IV and V are protected due to the inhibitory effect of ethanolic and aqueous extracts of *Premna serratifolia* respectively.

Hence it is obvious from this study that the leaves of *Premna serratifolia* is capable protecting the liver against hypercholesterolemia in damage. Alteration of architecture of liver tissue in the conditions of elevated lipid levels have been documented in the literature. The high cholesterol diet fed rats shows fatty cytoplasmic vaculated cells as compared to normal control. Treatment with aqueous extract of *T.chebula* shows less fatty cytoplasmic vacuoles as compared to high cholesterol diet fed rats. Combination of *T. chebula*along with high cholesterol diet shows focal area of cytoplasmic vacuoles (Dipa *et al*., 2010).

Conclusion

Hyperlipidemia though causes clinical manifestations, can be managed if properly handled. The present study reveals that the extracts of *Premna serratifolia* is capable of bringing down the elevated levels of lipid parameters like LDL, TG and Cholesterol. Hence it requires that further work need to be carried out using human subjects, such that the results can be extrapolated to human beings. It is also necessary to find the dose of the extract required for human use.

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