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Research Article

ANTI HYPERLIPIDEMIC EFFECT OF AQUEOUS EXTRACT OF *AEGLE MARMELOS* AND *CAMELLIA SINENSIS* IN OIL FED HYPERLIPIDEMIC RATS

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ABSTRACT

Objective: Hyperlipidemia is a serious health problem and greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body. The present research was carried out to find the inhibitory activity of HMGCoA reductase, ACAT (Acyl coenzymeA cholesterol acyl transferase) and antihyperlipidemic effect of *Aegle marmelos* and *Camellia sinensis* in oil fed rats.

Methods: The aqueous leaf extract of *Aegle marmelos* and *Camellia sinensis was* administered to the control and experimental rats for 21 days. After the experimental period the animals were sacrificed and the serum, tissue samples were used for its enzyme inhibitory activity and lipid metabolism analysis by using standard protocol.

Results: HMGCoA reductase is a key enzyme which continued the rate limiting step of cholesterol biosynthesis. Treatment with herbal drugs like *Aegle marmelos* and *Camellia sinensis* showed a significant decrease in the ACAT, HMGCoA reductase levels and significant increase in high density lipoprotein level. After treatment with *Aegle marmelos* and *Camellia sinensis* the histopathology results of liver proves the promising effect againt hyperlipidemia.

Conclusion: It is concluded that the aqueous leaf extract of *Aegle marmelos* and *Camellia sinensis* can be applied clinically for the management of hyperlipidemia and cardiovascular complications.

Keywords: Antihyperlipidemia, *Aegle marmelos, Camellia sinensis*, HMGCoA reductase, ACAT, lipoproteins.

INTRODUCTION

Hyperlipidemia is a major contributor towards many chronic non- infectious diseases like atherosclerosis, diabetes, Myocardial Infarction (MI), angina, stroke etc. Dyslipidemia are mainly related to some genetic variations in lipid metabolism or dietary habits or both which are highly prevalent in Indian sub-continent [1].

It is an important risk factor in the initiation and progression of atherosclerosis and Coronary Heart Disease (CHD). Medicinal plants have played a significant role in various ancient traditional systems of medicine. They are rich sources of bioactive compounds and thus serve as an important raw material for drug production and have become a target for the search of new drugs.

Aegle marmelos leaves contain sitosterol, aegelin, lupcol, rutin, marmesinin, eugenol, β- sitosterol, flavon, glycoside, montanine, oisopentenyl halfordiol marmelin and phenylethyl cinnamamides [2]. The active constituent of the fruit is marmorosin, which is identical to imperatorin. Roots of the tree have been found to contain psoralin, xanthotoxin, scopoletin and tembamide [3].

Green tea catechins have been suggested to have antiobesity effects. Experiments in rodent models demonstrated that tea catechins produced acute increases in fat oxidation and reduced dietary fat-induced weight gain [4]. *Camellia sinensis* ingestion decreases LDL cholesterol. Concurrently, HDL cholesterol increases, showing that green tea polyphenols exert an antiatherosclerotic effect. These results demonstrate that long-term feeding of tea catechins can be beneficial in the suppression of high-fat diet–induced obesity by modulating lipid metabolism.

Tea supplemented with vitamin E, administered to male hamsters, reduced plasma LDL cholesterol concentrations, LDL oxidation, and early atherosclerosis compared to the consumption of tea alone by the hamsters [5]. By this mechanism, green tea could possibly reduce the risk of associated diseases, including diabetes and coronary disease [6].

MATERIALS AND METHODS

Plants

Aegle marmelos and *Camellia sinensis* used in the study were procured from natural herbal shop in Tiruchirapalli and Theni respectively.

Preparation of extract

Fresh leaves of *Aegle marmelos* and *Camellia sinensis* were shade dried ground well until it becomes into a coarse powder. About 100g of coarsely powdered plant material was extracted with 250ml of water for 48 hours by maceration [7, 8]. Water extract thus obtained was filtered and vacuum dried using vacuum flash evaporator to yield the solid residue.

Hyperlipidemic induction

Mixture of pure coconut oil and ground nut oil (1:1) was used as the hyperlipidemic inducer in rats [9]. The volume of oil used was 8 ml/kg body weight/day for 28days to induce hyperlipidemic condition.

Animals

Albino Wistar male rats, weighing 100-150g were obtained from the institutional animal house, Tamil University, Thanjavur for the present investigations. The animals were housed at a room temperature of 25±2^{0}C, relative humidity of 75±5% and 12hrs dark-light cycle; animals were fed with standard laboratory diet and water ad libitum. The study was approved by IAEC and the experiments were conducted according to the ethical norms and Institutional Animal Ethics Committee Guidelines.

Procurement of diagnostic kits

Diagnostic kits used for the estimation of HDL, LDL, Total cholesterol, triglycerides were obtained from Agappe diagnostics Limited, Kerala. The reagents used for other estimations were purchased from Southern India chemicals, Tiruchirappalli and were of analytical grade.

Dosage preparation

Aegle marmelos and *Camellia sinensis* extracts were dispersed in saline and was administered orally at a dose of 250 mg/ kg body weight to the experimental rats for 28 days [7, 8].

Experimental design

Animals were divided into four groups and each group contains four rats. Group I rats orally treated with saline, group II rats orally received mixture of coconut oil and ground nut oil (8ml/100g b.w), group III rats orally received mixture of coconut oil and ground nut oil (8ml/100g b.w) and treated with *Aegle marmelos* (250mg/kg b.w) orally,group IV rats orally received mixture of coconut oil and ground nut oil (8ml/100g b.w) and treated with *Camellia sinensis* (250mg/kg b.w) orally for 28 days. At the end of the experiment, all the rats were sacrificed by jugular vein cut. Blood was collected, centrifuged and serum was separated. For plasma, blood was collected with anticoagulant and centrifuged (2000g for 20 min). The tissues were dissected out, weighed and washed using ice cold saline solution. Tissues were homogenized (10% w/v) in Tris-HCl buffer (0.1 M; pH 7.4) and centrifuged at 3000g for 20 minutes at 4°C. The resulting supernatant was used for various biochemical assays.

Biochemical estimations

Preparation of microsomes

Animals were sacrificed and the livers were removed, rinsed in cold normal saline and microsomes were prepared [10]. In brief, samples of liver were homogenized in 0.25M sucrose, 1mM EDTA, pH 7.2 and the homogenate were centrifuged at 10,000g for 10 minutes. The resultant supernatant was again centrifuged at 105,000g for 60mts and washed by resuspension. The final microsomal pellet was then resuspended in the same buffer.

Assay of ACAT activity

ACAT activity was done by the method Erickson *et al* [10]. To the assay mixture 150µg microsomal protein was added. Then 5nmol of ^{14}C-Oleoyl coenzymeA was added to initiate the reaction. The assay was terminated after 4minutes by the addition of chloroform/methanol (2:1) followed by ^{3}H-Cholesteryl oleate(8000 dpm) as an internal standard and read at 340 nm.

HMG CoA Reductase

Liver was removed and placed into ice-cold 0.25M sucrose solution. It was homogenized in 4 ml/g wet weight of tissue in ice-cold 0.25 M sucrose solution using a homogenizer. The crude homogenate was centrifuged for 20 min at 11,500 RPM and the pellets were discarded. Supernatant was collected and 0.1 ml 88 mM $CaCl_2$ was added per ml of supernatant. The supernatant was placed on ice for 5 min with occasional shaking. Then the supernatant was centrifuged at 13,500rpm for 35 min. The pellets were resuspended in 10 ml of 0.1 M Tris buffer, at pH 7.4 by homogenization, and it was stored at 20°C until use [11].

HMGCoA and NADPH are converted to Mevalonate and NADP respectively with the help of HMGCoA reductase. Rate of oxidation of NADPH to NADP is measured at 340 nm continuously at 30 sec intervals for 5 min. Inhibition of HMGCoA reductase decreases the rate of oxidation of NADPH. All reagents were prepared in 0.1 M Tris HCl buffer. All the procedure was carried out at 25^0 C. In brief 50µM HMGCoA and 10 mM Dithiothreitol (DTT) were preincubated for 1.5 hrs. For analysis, 0.1 M Tris HCl buffer, 1 mM Disodium EDTA, 75mM NaCl, and 50µl ml microsomes were incubated for 5 min at 25^0C. To this 0.1 mM NADPH and preincubated 50µM HMG CoA & 10 mM Dithiothreitol (DTT) were added and oxidation of NADPH was immediately recorded at 340 nm continuously at 30 sec intervals for 5 min [12].

Lipid profile: Total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, Triglyceride was determined using kit method.

Atherogenic Index (AI)

Atherogenic Index was calculated by using the following formula [13].

AI= Total serum cholesterol/ Total HDL cholesterol.

Histopathological Evaluation

The liver tissue samples were fixed in neutral buffered formalin for 24 hours. Sections of tissue from liver were examined histopathologically to study the anti-hyperlipidemic activity of *Aegle marmelos* and *Camellia sinensis*. The processed tissues were embedded in paraffin blocks and about 5 µm thick sections were cut using an American optical rotary microtome. These sections were stained with haemotoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, haemorrhage, edema and erosions using an arbitary scale for the assessment of severity of these changes.

Statistical analysis

The data obtained in present investigation was subjected to statistical analysis. The results are expressed as Mean ± S.D. The data was analyzed using SPSS software version (10.0).

RESULTS

Table 1: Changes in the level of ACAT and HMG-CoA reductase

Parameters	Group I	Group II	Group III	Group IV
ACAT*	56.82 ± 5.78	91.26 ±5.41[a] (60.61%)	67.10 ± 4.57[b] (-26.47%)	41.52 ± 3.34[c] (-54.5%)
HMG-CoA reductase*	25.62 ±1.37	53.01± 1.26[a] (106.9%)	34.47 ± 0.75[b] (-34.97%)	41.39 ±1.90[c] (-21.92%)

a - Significant different from Group I (*p<0.001*); b- Significant different from Group II (*p<0.001*)

c - Significant different from Group II (*p<0.001*)

*- Units/ mg protein

Group I- Normal rats; Group II- Oil induced rats

Group III –Induction of oil and *Aegle marmelos*; Group IV - Induction of oil and *Camellia sinensis*

Table- 1 gives the changes in ACAT and HMG CoA reductase levels. There was a significant increase in the ACAT (60.61%) and HMG CoA reductase levels (106.9%) in oil induced group compared to normal. Treatment with herbal drugs like *Aegle marmelos* (-26.47%; -34.97%) and *Camellia sinensis* (-54.5%; -21.92%) showed a significant decrease in the ACAT and HMG CoA reductase levels.

Group I- Normal rats; Group II- Oil induced rats

Group III –Induction of oil and *Aegle marmelos*; Group IV - Induction of oil and *Camellia sinensis*

a – Significant different from Group I (*p<0.001*); b- Significant different from Group II (*p<0.001*)

c - Significant different from Group II (*p<0.001*); NS*- Non significant from Group II (*p<0.1*)

NS**- Non significant from Group II (*p<0.1*)

*- mg/dl; #- %

Table 2 gives the changes in Total cholesterol, HDL, LDL, VLDL, TGL, AI, CRI levels. There was a significant increase in the Total cholesterol (53.08%), LDL (228.11%), VLDL (46.93%), TGL (95.22%), AI (175.31%), CRI (122.91%) and decrease in HDL (-39.2%) levels in oil induced group compared to normal. Treatment with herbal drugs like *Aegle marmelos* and *Camellia sinensis* showed a significant decrease in the Total cholesterol (24.76%; -31.26), LDL (-55.49%; -74.2%), VLDL (-8.89%; -20.14%), TGL (-26.05%; -30.07%), AI (-53.78%; -75.73%), CRI (-45.58%; -63.11%) and increase in HDL (61.2%, 71.04%) levels.

Table 2: Changes in the level of total cholesterol, HDL, LDL, VLDL, TGL, AI, and CRI

Parameters	Group I	Group II	Group III	Group IV
Total cholesterol*	161.75 ± 7.67	293 ± 2.16[a] (53.08 %)	202.5 ± 4.43[b] (-24.76%)	151 ± 6.97[c] (-31.26%)
HDL*	44 ± 2.58	35.25± 1.89[a] (-39.2%)	44.75 ± 3.30[b] (61.2%)	49 ± 3.16[c] (71.04%)
LDL*	103.6 ± 3.53	229.3± 3.64[a] (228.11 %)	135.4 ± 7.07[b] (-55.49%)	79.75 ± 6.18[c] (-74.2%)
VLDL*	15.65 ± 0.59	28.4 ± 1.14[a] (46.93 %)	22.35 ± 1.14[NS*] (-8.89%)	22.15±1.57[NS**] (-20.14%)
TGL*	78.25 ± 2.98	143 ± 5.71[a] (95.22 %)	111.75 ± 5.73[b] (-26.05%)	110.75±7.88[c] (-30.07%)
AI#	2.35 ± 0.20	6.47 ± 0.40[a] (175.31 %)	2.99 ± 0.37[b] (-53.78%)	1.57 ± 0.12[c] (-75.73%)
CRI#	3.71±0.22	8.27±0.47[a] (122.91 %)	4.5±0.40[b] (-45.58%)	3.05±0.17[c] (-63.11%)

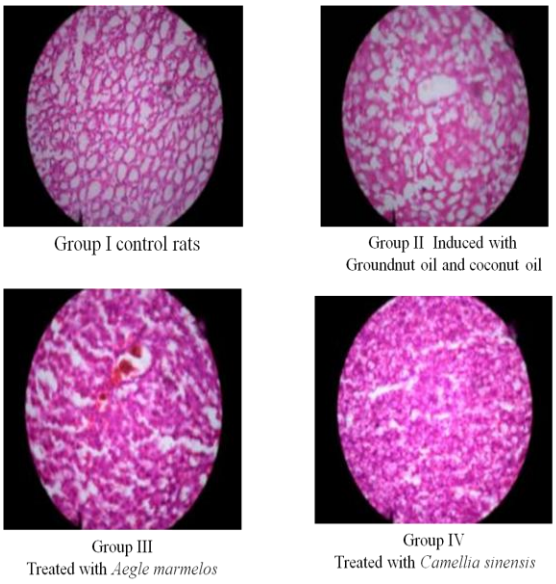

Fig. 3: Histopathological evaluation of liver in control and experimental rats

Fig-3 indicates the histopathological observation of control and experimental rats. The liver section of control group showed normal arrangement of hepatocytes with clear broad central vein at portal layer. The accumulation of fat was increased in the hyperlipidemic control and large area of hepatocytes taken over by macro droplet of fat. Treatment with leaf extracts of *Aegle marmelos* and *Camellia sinensis* shown micro droplet of fat accumulation on rat histopathology.

DISCUSSION

Hyperlipidemia (elevated levels of triglycerides or cholesterol) and reduced HDL-C occurs as a consequence of several interrelated factors that may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism [14]. Elevated serum concentration of total cholesterol, LDL-C appears to increase the risk of an individual in developing Coronary Heart Disease (CHD). Lipid lowering therapy is indicated in the primary and secondary prevention of CVD in addition to the management of all other risk factors including smoking, diabetes and obesity [15].

The level of ACAT and HMG-CoA reductase was significantly elevated ($p<0.001$) in the oil induced hyperlipidemic rats when compared to the control. Administration of coconut and groundnut oil provides large amount of substrate availability to ACAT and HMG-CoA reductase. So the levels of ACAT and HMG-CoA reductase were significantly elevated in the induction group. Treatment with *Aegle marmelos* and *camellia sinensis* had shown significant reduction ($p<0.001$) and brought back the level of these enzymes to near normal. It was reported that the presence of saponins in *Aegle marmelos* and catechin in *Camellia sinensis* reduce the level of ACAT and HMG-CoA reductase by competing with cholesterol at its binding sites or interfering with cholesterol biosynthesis in the liver or inhibiting HMG-CoA reductase and ACAT [16].

There was significant increase in the level of TG, LDL, VLDL ($p<0.001$) and reduction in the level of HDL ($p<0.001$) in the oil induced rats when compared to control. Elevating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis [17].

On treatment with drug *Aegle marmelos* and *camellia sinensis*, the level of TG, LDL, VLDL ($p<0.001$) and HDL ($p<0.001$) was significantly brought back to their near normal levels. Basic mechanism behind the reduction in the level of TG, LDL, and VLDL and increased in the level of HDL was due to the breakdown of lipids by plant drugs, thus modifying the altered lipid metabolism [18]. Increase in HDL levels and reduction in LDL, VLDL and TG shows the intensive conversion of LDL to HDL and clearance of circulating lipids. It was reported that the presence of high amounts of saponins, tannin, eugenol, marmesinin, marmelosin, cuminaldehyde, aegelin, psoralen, marmin in *Aegle marmelos* [19] and catechins, theobromine, flavanoids, polyphenols in *camellia sinensis* [20] might contribute to the reduction of cholesterol, by increasing repel of faecesic estrol and increased peristaltic movement [21]. Similar result was already reported [15]. So reduction in the lipoproteins and cholesterol levels decrease the further complication and protect the cell from free radical induced damage [22]

In our present study, histopathological examination of the liver of control rats showed normal hepatic cell architecture. But histopathological results of hyperlipidemic induced group (groundnut and coconut oil treated rats) showed marked fatty infiltration of the liver along with the dilatation of cyanosoides. Treatment with *Aegle marmelos* and *Camellia sinensis* showed mild changes in the hepatic cell, thus showing congestion similar to normal architecture, which indicates the protective effect of *Aegle marmelos* and *Camellia sinensis*.

The present study indicated the protective effect of *Aegle marmelos* and *Camellia sinensis* on hypercholesterolemia and the noticed effect may be due to its active components. However, further research is needed at the molecular level to establish the present findings.

CONCLUSION

By performing the above work, it can be concluded that the leaf extracts of *Aegle marmelos* and *Camellia sinensis* possess anti-hyperlipidemic effect by controlling the enzymes HMGCoA reductase and ACAT. Since the effect of drugs were highly beneficial without any side effects, the research about the *Aegle marmelos* and *camellia sinensis* can be further extrapolated to the humans for the service of our society.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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