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# HYPOLIPIDEMIC AND ANTI OXIDANT ACTIVITY OF RUELLIA TUBEROSA Linn.

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## ABSTRACT

*Objective: To study the efficacy of Ruellia tuberosa ethanolic extract (RTEE2012) in reducing the cholesterol levels and as an antioxidant in hypercholesterolemic rats. Materials and Methods: Hypercholesterolemia was induced in normal rats by including high fat diet (cholesterol 25mg/kg in oil). Powdered form of RTEE2012 was administered as feed supplement at 250, 500 and 1000 mg/kg dose levels to the hypercholesterolemic rats. Plasma lipid profile, hepatic superoxide dismutase (SOD) activity, catalase activity and extent of lipid peroxidation in the form of malondialdehyde were estimated using standard methods. Results: Feed supplementation with 250, 500 and 1000 mg/kg of RTEE2012 resulted in a significant decline in plasma lipid profiles. The feed supplementation increased the concentration of catalase, SOD and HDL-c significantly in the experimental groups (250, 500 and 1000 mg/kg). On the other hand, the concentration of malondialdehyde, cholesterol, triglycerides, LDL-c and VLDL in these groups (250, 500 and 1000 mg/kg) were decreased significantly. Conclusion: The present study demonstrates that addition of RTEE2012powder at 250, 500 and 1000 mg/kg level as a feed supplement reduces the plasma lipid levels and also decreases lipid peroxidation.*

## KEYWORDS

*Antioxidant, in vivo, RTEE2012, lipids, Atorvastatin*

## 1. INTRODUCTION

*One of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases is Hyperlipidemia.[1] Hyperlipidemia is characterized by elevated serum total cholesterol, low density, and very low-density lipoprotein and decreased high-density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease.[2] The primary cause of death is Coronary heart disease, stroke, atherosclerosis and hyperlipidemia.[3] Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.[4] The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease.[5] The treatment of hyperlipidemia depends on the patient's cholesterol profile. Many anihyperlipidemic agents like statin, fibrates, niacin, bile acids, ezitimibe etc reduce cholesterol level with different condition.[6]*

*Currently available hypolipidemic drugs have been associated with a number of side effects.[7]The*

consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function.[8] Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of hyperlipidemia in many parts of the world. There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine. Recently, the search for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs.[9]Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated by anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia.[10]

Consumption of much fat may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Therefore, improvement in human diet is highly recommended for disease prevention[11]. Increase in the cholesterol level is a major risk factor for progression of atherosclerosis, which is usually accompanied by the production of free radicals. Patients with cardiovascular disease showed significant increases in lipid peroxidation, which correlates with severity of hypercholesterolemia.[1, 2]Recent scientific research strategies have been focusing on the removal of reactive oxygen species (ROS).[12] Lipid peroxidation is a key marker of oxidative stress results in extensive membrane damage and dysfunction.[13]Medicinal plants play a major role in hypolipidemic activity.

***Ruellia tuberosa L.*** belongs to the family of Acanthaceae. The common names are Cracker plant in English and Pattaskai in Tamil. ***Ruellia tuberosa L.*** is a tropical perennial plant with a hairy quadrangular stem growing up-to a height of 6.5 cm. The leaves are simple, opposite elliptic about 5cm in length. The plant flowers only after the start of the rainy season. The flower is bisexual and violet in color. The capsule contain 7 to 8 seeds each burst and open with a bang when they get wet and the black seeds are hurdled away. The capsules are baton shaped and 3cm in length and turn black with the age. The plant has thick finger like roots and the plant prefers semi shady moist conditions. Whole plant of ***Ruellia tuberosa L.*** was used to treat bladder diseases and frequent micturition; decoction with Petiveria alliacea is drunk to "clean out" uterine tract (dilation and curettage) or as an abortifacient.[14, 15]

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Roots of Ruellia tuberosa was collected after authentication by Dr. K. Madhava chetty, Assistant

*professor, Department of botany, Sri venkateswara university, Tirupati, A.P, India. The plant was collected from the chittor forest; collected plant was washed thoroughly with water and dried in the shade. Ethanolic extract was obtained by extracting powder with 95%ethanol by soxhlet extraction method for 72hr. After completion of the extraction the solvent was removed by rotary evaporator method. The ethanolic extract was used for further study.*

### *2.2 Experimental Animals*

*The study was carried out after obtaining the Animal ethics committee approval (Reg. No. 769/2010/CPCSEA). Albino wistar rats, maintained at a 12 h light/dark cycle, were used for the study. Animals were housed under standard laboratory conditions, with free access to food and water, ad libitum.*

### *2.3 Chemicals*

*Cholesterol, coconut oil, all chemicals used including solvents was of analytical grade.*

### *2.4 Experimental Design*

*Acute oral toxicity study was performed using albino mice as per OECD (Organization for Economic cooperation and development) guidelines. Ruellia tuberosa was found to be safe up to 2000mg/kg body weight when administered orally. Three doses were selected for the study 250mg/kg, 500mg/kg and 1000mg/kg.*

***Evaluation of Hyperlipidemic activity:*** *Hyperlipidemia was induced by feeding a high fat diet (cholesterol 25mg/kg in oil) to healthy rats for 30days. Rats were divided into five groups containing six animals each.*

*Group 1 received normal diet (normal)*
*Group 2 received high fat diet (control).*[16]
*Group 3 received RTEE2012 250 mg/kg, p.o*[17]
*Group 4 received RTEE2012 500 mg/kg p.o.*
*Group 5 received RTEE2012 1000 mg/kg p.o.*
*Group 6 received Atorvastatin 10 mg/kg p.o.*[18]

*A homogeneous solution of the extracts and standard drug atorvastatin was freshly prepared individually using 10% v/v DMSO. Rats were fed daily with standard diet supplied by cholesterol in oil was given by oral route at 10 am and Ruellia tuberosa extracts or Atorvastatin was given by oral route at 3 pm daily, to respective groups, for a period of 30 days. The normal control group was treated with vehicle instead of drugs. Initial and final body weights and food intake of rats were monitored. At the end of the experimental study, animals were fasted for 12 hr and blood samples were collected by retro-orbital puncture technique in a coagulant-free vessel, and were kept at room temperature for 1 h. Samples were centrifuged at 4000–5000 rpm to separate serum, which was subjected for the estimation of lipid profile.*[19]*Immediately after sacrificing the animals, livers were separated, washed with pH 7.4 buffer, blotted with dry filter paper. A part of the liver was minced and then homogenized in pH 7.4 buffer and*

*was used for the estimation of lipid peroxidation. Another part of the liver was minced and then homogenized in pH 7 buffer and was used for the estimation of Superoxide dismutase.*

***The main parameters assessed in hyperlipidemic model were as follows:***

*Biochemical lipid constituents/parameters — The main biochemical parameters recommended by the National Cholesterol Education Program (NCEP) guidelines (2002) for lipid screening ie. Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL), Very Low Density Lipoprotein Cholesterol (VLDL), High Density Lipoprotein Cholesterol (HDL) and Triglycerides (TG) were evaluated from the serum.*

*Cardiac risk indicators — The cardiac risk ratios recommended by NCEP guidelines (2002) were estimated by calculating the TC: HDL ratio (Atherogenic Index) and LDL: HDL ratio.*

***Evaluation of Antioxidant activity:***

*A) Estimation of Lipid peroxidation assay:*

*The tissues were homogenized in 0.1 M phosphate buffer pH 7.4 with Tissue homogenizer. Lipid peroxidation in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily. 0.2 ml of tissue homogenate, 0.2 ml of 8.1 % of sodium dodecyl sulfate (SDS), 1.5 ml of 20 % acetic acid and 1.5 ml of 8 % TBA were added. The volume of mixture was made up to 4 ml with distilled water and then heated at 95oC on water bath for 60 min using glass ball as a condenser. After incubation, tubes were cooled to room temperature and final volume was made to 5 ml in each tube. 5 ml of butanol: pyridine (15: 1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its O.D. read at 532 nm against an appropriate blank without the sample. The level of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56 × 105 Ml/cm.*[20]

*B) Estimation of super oxide dismutase (SOD):*

*0.5ml of sample was diluted with 0.5ml distilled water, to this 0.25 ml ethanol, 0.5ml of chloroform (all chilled reagents) were added. The mixture was shaken for 1min and centrifuged at 200 rpm for 20 min. The enzymatic activity of supernatant was determined. To it 0.05ml of carbonate buffer (0.05M pH10.2) and 0.5ml of EDTA (0.49M) was added. The reaction was initiated by addition of 0.4 ml epinephrine (3mM) and the change in optical density/mm was measured at 480nm. SOD was expressed as units/mg. Protein change in optical density/mm.50% inhibition of epinephrine to adrenochrome transition by enzyme is taken.*[20] *Absorbance was measured spectrophotometrically at 480nm.* [20]

*C) Estimation of catalase:*

*A 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7). Reaction was started by the addition of 1 ml of freshly prepared 30 mM $H_2O_2$. The rate of decomposition of $H_2O_2$ was measured spectrophotometrically from changes in*

*absorbance at 240 nm. Activity of catalase was expressed as unit/mg protein.* [20]

***Statistical analysis***

*All data are presented as mean ± SEM. Statistical analysis is done to investigate the relationship among the groups using one-way ANOVA. The P values ≤ 0.001 were considered as statistically significant.*

## *3. RESULTS*

*Addition of RTEE2012 as a feed supplement at three doses, i.e.250, 500, 1000 mg/kg, resulted in a dose-dependent reduction in lipid profiles in plasma along with significant reduction in lipid peroxidation.*

| S. NO | GROUP | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | TG (mg/dl) | TC (mg/dl) | LDL : HDL | ATHEROGENIC INDEX |
|---|---|---|---|---|---|---|---|---|
| 1 | NORMAL | 43.88± 1.79 | 17.42±1.61 | 19.62±0.42 | 98.12±3.72 | 80.12±1.48 | 0.404 | 0.357 |
| 2 | HFD | 19.59±1.59 | 94.63±1.16 | 22.48±0.35 | 112.4±1.59 | 136.7±1.76 | 4.83 | 0.758 |
| 3 | HFD+ RTEE DOSE I | 42.8 ±3.91 | 65.67±3.88 | 21.83±1.48 | 109.16±1.45 | 130.3±2.82 | 1.534 | 0.406 |
| 4 | HFD+ RTEE DOSE II | 49.3±2.97 | 54.12±1.54 | 21.25±0.63 | 106.28±2.62 | 124.67±2.38 | 1.097 | 0.332 |
| 5 | HFD+ RTEE DOSE III | 52.6±0.78 | 37.14±1.23 | 20.8±0.51 | 104.02±0.60 | 110.54±1.24 | 0.706 | 0.296 |
| 6 | HFD+ STD | 53.38±0.92 | 34.15±1.44 | 17.95±0.37 | 89.75±0.37 | 105.48±2.16 | 0.639 | 0.295 |

***Table 1: TC – Total Cholesterol, TG – Triglycerides, LDL – Low density lipoprotein, HDL – High density lipoprotein, VLDL – Very low density lipoprotein. n = 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P ≤ 0.001.Variation analysis done by One way ANOVA by comparing of RTEE2012 with control group.***

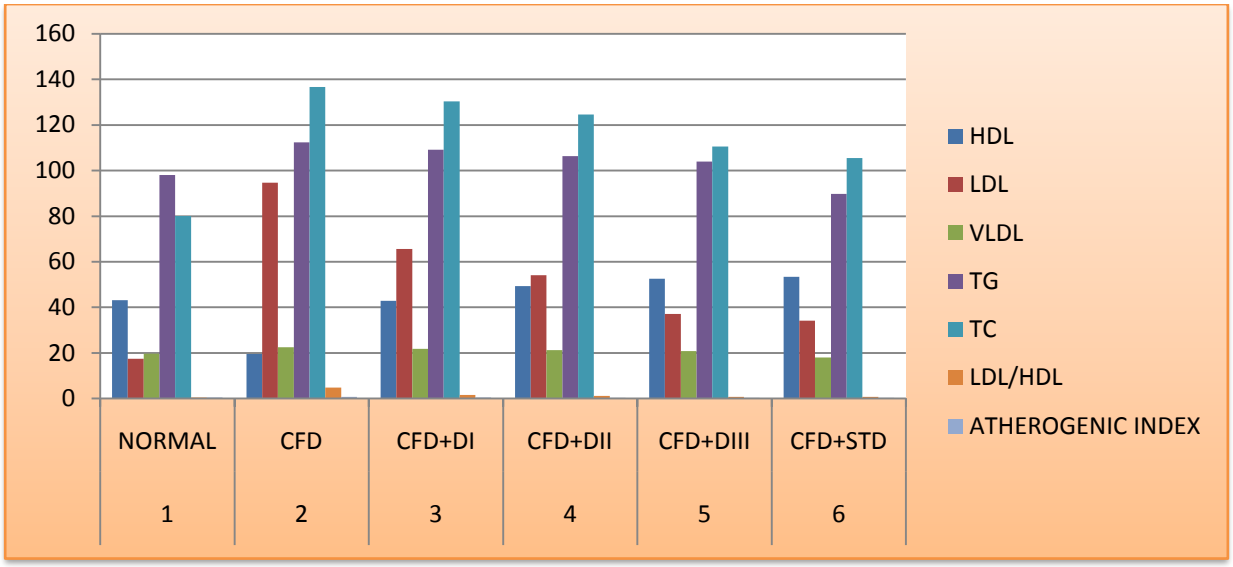


***Figure 1 and Table 1 shows that RTEE2012, the total lipids i.e. total cholesterol and triglycerides in plasma as well as LDL and VLDL cholesterol were significantly reduced at both doses of feed supplementation. However, HDL cholesterol level increased in both treated groups significantly. This observation indicates that, as a feed component is effective in reducing plasma LDL and VLDL levels. It is well known that increased HDL levels have a protective role in CAD.***

| S.NO | GROUP | LPO | SOD | CAT |
|---|---|---|---|---|
| 1 | NORMAL | 30.45±0.61 | 7.92±0.56 | 65.24±2.01 |
| 2 | HFD | 48.74±0.67 | 4.35±0.27 | 40.55±0.39 |
| 3 | HFD+ RTEE DOSE I | 45.66±0.94 | 4.82±0.17 | 53.27±1.71 |
| 4 | HFD + RTEE DOSE II | 43.85±1.16 | 4.98±0.17 | 56.26±1.34 |
| 5 | HFD + RTEE DOSE III | 41.57±0.75 | 5.13±0.18 | 58.96±0.33 |
| 6 | HFD + STD | 39.24±0.49 | 6.24±0.33 | 67.26±1.25 |

***Table 2: LPO – Lipid peroxidation, CAT – catalase, SOD – superoxide dismutase. n = 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P≤0.001.Variation analysis done by One way ANOVA of RTEE2012 with control group***

***Table 2*** *shows that the RTEE2012 -treated groups have higher levels of anti oxidative parameters (catalase, superoxide dismutase) and decreased level of lipid peroxidation indicating its efficacy to reduce the LDL-c oxidation. Standard drug*

*atorvastatin, at a dose of 10 mg/kg of body wt. cause decreased serum cholesterol, triglyceride, LDL and VLDL levels, whereas HDL was increased more as compared to both doses of the test drug. The results of our study showed that administration of high fat diet induced significant production of MDA in liver, and administration of RTEE2012 significantly decreases the MDA production in liver. RTEE2012 also resulted in a significant increase in the liver CAT, SOD as compared to the control animals, which suggests its antioxidant activity.*

## *4. DISCUSSION AND CONCLUSION*

*Hypercholesterolemia, a high cholesterol diet and oxidative stress increase serum LDL levels resulting in increased risk for development of atherosclerosis.[21] Malondialdehyde a secondary product of lipid peroxidation is a major reactive aldehyde; higher levels can lead to peroxidation of biological membranes.[2.2] The antioxidant enzymes, mainly superoxide dismutase and catalase are first-line defensive enzymes against free radicals.[23] The qualitative analysis of RTEE2012 indicated the presence of tannins, flavonoids and phenols. It is well known that tannins, flavonoids and phenols are natural antioxidants but have also been reported to significantly increase SOD and catalase activities. Further, it was shown that these compounds act as promoters for SOD and catalase and cause the expression of SOD and catalase.[24] The currently noted elevated levels of SOD and catalase with RTEE2012 could be due to the influence of tannins, flavonoids and phenols. Lipid peroxidation is a free radical mediated process, which has been accepted to be one of the principle causes of cholesterol-induced diseases, and is mediated by the production of free radical derivatives.[25]*

*Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack.[26] The biochemical mechanisms involved in the development of hypercholesterolemia have long been investigated. MDA, a stable metabolite of the free radical mediated lipid peroxidation cascade, is widely used as marker of lipid peroxidation. Lipid peroxide levels in tissue were found to be significantly elevated in hypercholesterolemic rats.[27] The Catalase, SOD antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Roots were used to treat kidney diseases; syrup of root was used to treat whooping cough; infusion or decoction for a diabetes remedy; plant part namely tubers is used as tea for cleansing the blood. The roots and leaves are used as tea for alleviating the retention of urine and to remedy weakness. Leaf contains apigenin and luteolin. Seed oil yields myristic, capric and lauric acids. The plant tuber has ethno medicinal uses in relieving abdominal pain after delivery. In folk medicine, **Ruellia tuberosa L.** has been used as anti-diabetic, antipyretic, analgesic, anti hypertensive, thirst quenching, antidotal agent and this plants was traditionally used for reducing*

*toxicity, healing urine tract inflammation. This plant has antimicrobial activity for both Gram positive and Gram negative bacteria. However, very few chemical constituents and pharmacological activities have been reported for this species. Therefore it is prudent to look for options in herbal medicine for major chronic diseases.*[28]

*To conclude, feed supplementation with RTEE2012 reduced the hyperlipidemic and oxidative conditions. RTEE2012 appears to ameliorate hypercholesterolemia probably by decreasing the exogenous cholesterol absorption and increasing the endogenous cholesterol conversion to bile acid, though to find out the exact mechanism further studies are needed.*

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