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ANTIHYPERLIPIDEMIC OF RUELLIA TUBEROSA LINN IN TRITON INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. The present study was designed to investigate the antihyperlipidemic activity of Ruellia tuberosa ethanolic extract (RTEE 2012) in Triton X-100 induced Hyperlipidemic rats. RTEE 2012 was administered at a dose of 250, 500 and 1000mg/kg, (p.o) to Triton induced Hyperlipidemic rats. Atorvastatin is used as reference standard. The statistical analyses were carried out using one way ANOVA. RTEE 2012 show a significant decrease in the levels of serum cholesterol, triglycerides, LDL, VLDL and significant increase in the level of serum HDL with increase in dose of RTEE 2012 against Triton induced hyperlipidemic rats. Therefore it effectively suppressed the Triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

Keywords: RTEE 2012, Hyperlipidemia, Triglycerides, lipoprotein, Triton X-100

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [1]. Reactive oxygen species induced depletion of antioxidants plays a main role in the initiation of atherosclerosis and the development of CVD [2]. Increased formation of free radicals/reactive oxygen species (ROS) contributes to cardiovascular disease (CVD) progression [2, 3].

The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification, and DNA breaks [4]. Reactive oxygen species induce cardiac dysfunction and cardiac apoptosis and/or necrosis in heart failure [5]. Reactive oxygen species are formed intracellularly and are controlled by antioxidant defense. World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Hyperlipidemia is a metabolic disorder, specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), and Triglycerides (TAG) with a concomitant decrease in the concentrations of High Density Lipoprotein Cholesterol (HDL-C) in the blood circulation [6]. The use of complimentary/alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide in these days. Currently available drugs have been associated with 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]. The study mainly focuses to reduce the risk of developing ischemic heart disease or the

occurrence of further cardiovascular disease or cerebrovascular disease in patients with hyperlipidemia [9].

***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 [10, 11].

MATERIALS AND METHODS

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 chituru forest; collected plant was washed thoroughly with water and dried in the shade. Ethanolic extract was obtained by extracting powder with95%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.

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*.

Chemicals: Triton X-100 (a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer). Atorvastatin was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

Experimental Design: Acute oral toxicity study was performed using albino mice as per OECD (Organization for Economic cooperation and development) guidelines [12]. 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 .

Induction of Hyperlipidemia: Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h [13].The animals were divided into four groups of six rats each. The first group was given standard pellet diet, water and orally administered with 10%DMSO. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 10%DMSO (p.o) for 7 days. The third group was administered a daily dose of RTEE 2012 250mg/kg dissolved in 10%DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fourth group was administered a daily dose of RTEE 2012 500mg/kg dissolved in 10%DMSO, p.o., for 7 days, after inducing hyperlipidemia. The fifth group was administered a daily dose of RTEE 2012 1000mg/kg dissolved in 10%DMSO, p.o., for 7 days, after inducing hyperlipidemia. The sixth group was administered with the standard Atorvastatin 10mg/kg, p.o. for 7 days [14].

Collection of blood: On the 8thday, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed. A part of liver sections were isolated and preserved in 10% formalin [15]. The liver sections were evaluated for histopathology to assess any architectural changes.

Biochemical analysis: The serum extract was assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods [16].

Statistical analysis: The results were expressed as mean $\pm$ S.E.M. Statistical analysis was carried out by using one way ANOVA. Statistically significant at $P \leq 0.001$. Variation analysis is done by one way ANOVA comparing RTEE 2012 with control group.

RESULTS

In triton induced hyperlipidemic model, the groups treated with the RTEE 2012 and Atorvastatin demonstrated a significant decrease in the serum TC,

LDL, VLDL, TG, besides an increase in serum HDL levels when compared to triton induced hyperlipidemic control group (Table 1and Table 2). The groups treated with the RTEE 2012 and Atorvastatin demonstrated significant decrease in the Atherogenic Index and LDL: HDL risk ratios. The groups treated with the RTEE 2012 also showed decrease in body weights when compared to triton induced hyperlipidemic control group. In triton induced hyperlipidemic model, the histopathological studies were conducted in the liver sections of rats and the histopathological changes were observed (Fig. 1). These figures illustrate the protective action of the RTEE 2012 against fatty infiltration and granular degeneration due to hyperlipidemia closely comparable to that with Atorvastatin. The RTEE 2012 showed a significant antihyperlipidemic activity in the animal model and the best activity was shown by RTEE 2012.

The ethanolic extract of Ruellia tuberosa was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. The Phytochemical tests with the RTEE 2012 indicated the presence of tannins, phenols, and flavonoids.

DISCUSSION AND CONCLUSION

Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values has been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the lipid profile in serum. Lipid profile in serum indicates that increased triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 1g/day RTEE 2012. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Table 1 and Table 2. The RTEE 2012 markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, VLDL, LDL and the reduction in the HDL level. It can be concluded that 1g/day of RTEE 2012 treatment was effective in reduction of cholesterol, TG, VLDL, LDL and HDL in a dose dependant manner. The histopathological findings in the liver of rats fed with the RTEE 2012 showed decrease in granular degeneration caused by triton. All these beneficial effects of the extracts may be due to their antioxidant and antihyperlipidemic effects carried out by tannins, phenols, flavonoids.

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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 $\leq$0.001.Variation analysis done by one way ANOVA comparing of RTEE 2012 with control group.

GROUP	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)	LDL/HDL
NORMAL	43.87±0.55	27.35±2.36	17.49±0.75	0.62
TRITON TREATED	28.31±3.33	94.78±1.82	33.74±0.87	3.34
TRITON+DOSE1	42.9±0.30	74.79±1.04	28.48±0.74	1.74
TRITON +DOSE2	49.59±0.86	60.79±0.88	26.12±0.24	1.22
TRITON+DOSE3	54.38±0.80	47.64±1.06	22.86±0.24	0.87
TRITON+STD	56.27±1.45	42.46±0.63	20.39±1.02	0.75

Table 2: TC – Total Cholesterol, TG – Triglycerides. 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 comparing of RTEE 2012 with control group.

GROUP	TG(mg/dl)	TC(mg/dl)	ATHEROGENIC INDEX
NORMAL	78.96±1.46	84.28±0.86	0.252
TRITON TREATED	160.24±1.15	152.58±0.89	0.752
TRITON+DOSE1	121.53±1.75	130.46±1.19	0.451
TRITON +DOSE2	106.49±0.94	118.68±1.07	0.330
TRITON+DOSE3	93.01±1.13	116.24±1.72	0.232
TRITON+STD	92.98±1.19	115.27±1.71	0.212

Figure 1: Histopathology of liver section of rats.

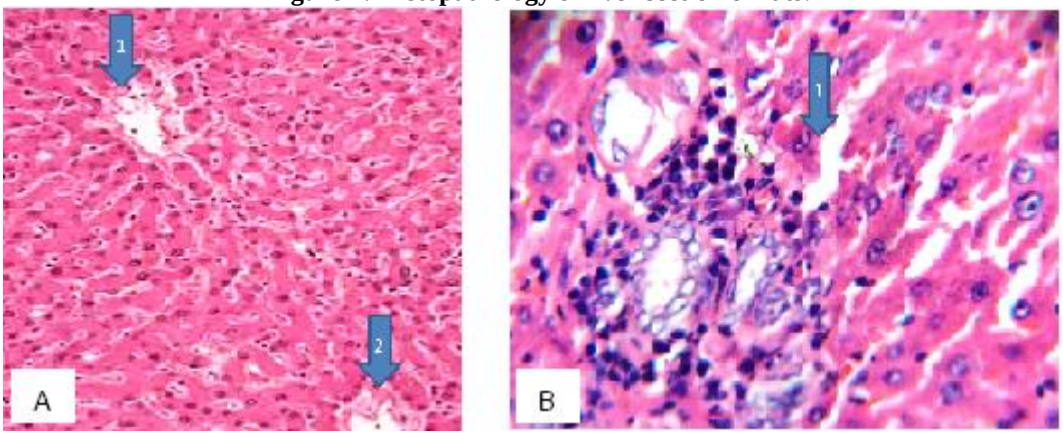

Fig A: Normal liver. (1: Portal tract, 2: Central venule)
Fig B: Standard dose. (1: Liver congestion, Mild periportal lymphocytic infiltration)

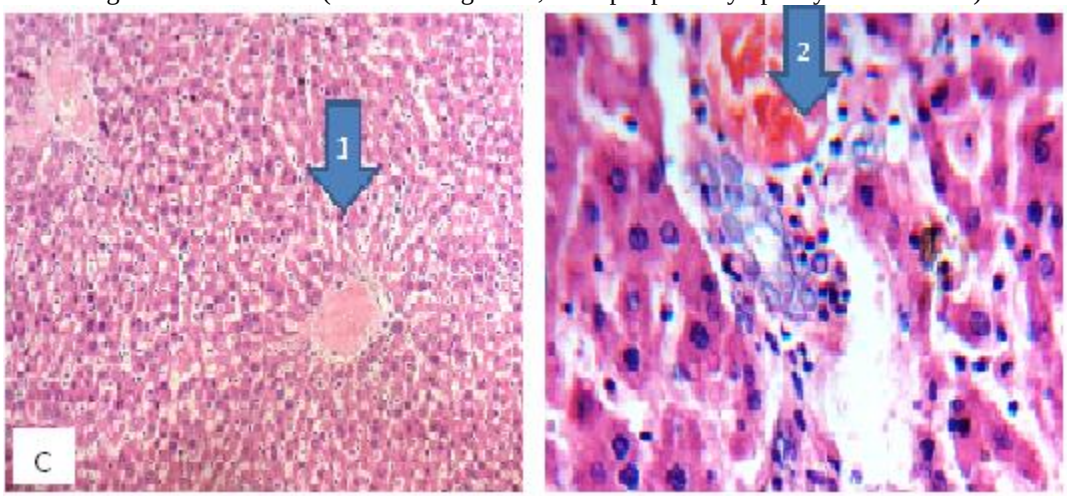

Fig C: Control dose. (1: Necrosis of liver cells in small groups around central veins, 2: Moderate periportal inflammation)

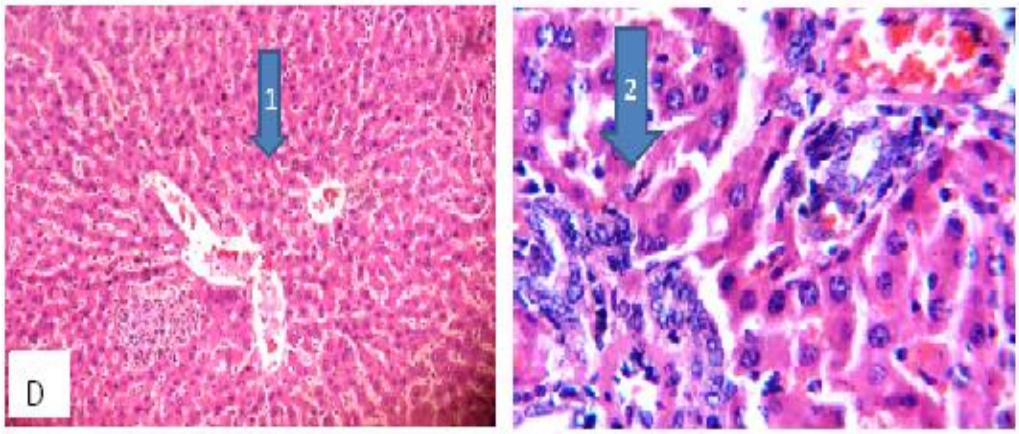

Fig D: RTEE 2012 dose1. (1: Focal necrosis of cells near central vein with inflammatory infiltrate ,mild edema, necrosis, 2: Mild periportal inflammation)

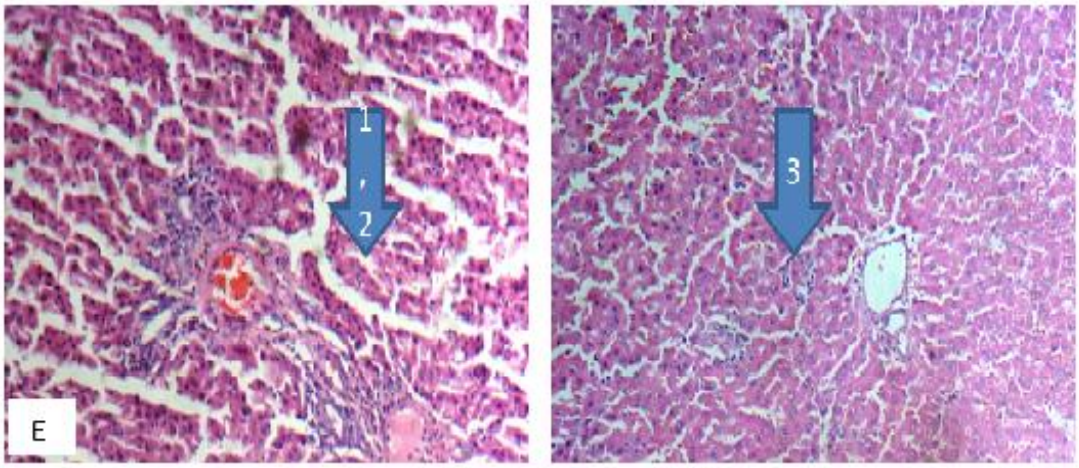

Fig E: RTEE 2012 dose2. (1: Focal necrosis of hepatocytes around central veins, 2: Congestion of sinusoids, 3: Mild periportal inflammation)

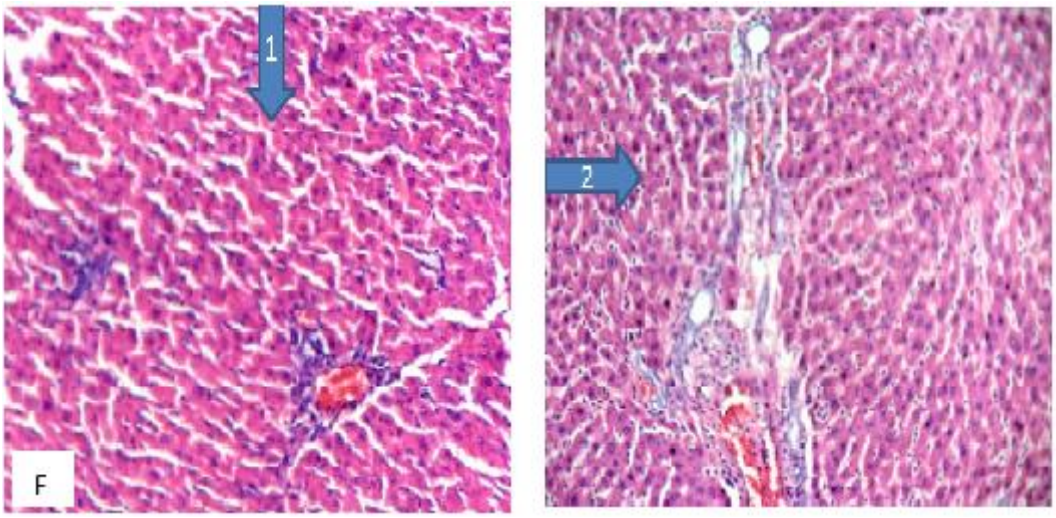

Fig F: RTEE 2012 dose3. (1: Liver within normal limits, 2: Mild Periportal inflammation)

REFERENCES

1. A.Y. Kaesancini, R.M. Rauss. Curr Opinion Lipidol, 1994; 5: 249-51.
2. Kaliora AC, Dedoussis GVZ, Schmidt H. Atherosclerosis, 2006; 18: 1.
3. Wattanapitayakul SK, Bauer JA. Pharmacol Ther, 2001; 89: 187.
4. Hiroi S, Harada H, Nishi H. Biochem Biophys Res Commun, 1999; 261: 332.
5. Griendling KK, Ushio-Fukai M. J Lab Clin Med, 1998; 132: 9.
6. Dhuley J, Naik SR, Rele S, Banerji A. Pharm Pharmacol Commun, 1999; 5: 689.
7. S.L. Brown. Br Med J, 1996; 313: 637-8.
8. G. Davey Smith, J. Pekkanen. Br Med J, 1992; 304: 431-40.
9. Fu-An C, An-Bang W, Pochuen S, Daih-Huang K, Chi-Ying H. Food Chem, 2006; 97: 654-60.
10. Chwan-Fwu L, Yu-Ling H, Lee-Ying C, ShuennJyi S, Chien-Chih C. J Chinese Med, 2006; 17: 103-9.
11. Ecobichon D J. The Basis of Toxicology Testing, 3rd Edition New York, CRC Press, 1997; 43.
12. Hicham hurnafi, *et al.* J Ethnopharmacol, 2007; 109: 156-60.
13. K. Muramatsu, M. Fukuyo, Y. Hara. J Nutr Sci Vitamin, 1986; 56:509-20.
14. Z.Y. Ding, Y. Chen, M. Zhou, Y.Z. Fang. Clin J Pharmacol Toxicol. 1992; 6:263-6.
15. Vogel G, Vogel WH. Influence of lipid metabolism. In: Drug Discovery and Evaluation Pharmacological Assay. Springer-Verly: Berloin; 1997. pp: 604-8.