INVESTIGATION AND EVALUATION OF HYPOLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF RUELLIA TUBEROSA ROOTS IN HIGH FAT DIET AND TRITON INDUCED HYPERLIPIDEMIA IN RATS

ANTHADUPULA SURESH *, *Dr. D SWATHI, Dr. R HEMALATHA*

Department of Pharmacology, Holy Mary Institute of Technology & Science Keesara, Bogaram, Ghatkesar Road, Kondapur, Telangana 501301

ABSTRACT : ***The aim of the study was to examine the possible hypolipidemic and antioxidant activities of ethanolic extract of Ruellia tuberosa roots in High fat diet (cholesterol 25mg/kg) and triton(100mg/kg) induced hyperlipidemia.The present study, "Investigation and evaluation of Hypolipidemic and antioxidant activities of ethanolic extract of Ruellia tuberosa Linn roots in high fat diet and triton induced hyper lipidemia in rats" was done with the following .The objectives are to investigate hypolipidemic activity of ethanolic extract of Ruellia tuberosa in,High fat diet (cholesterol: 25mg/kg) induced hyperlipidemia in rats,Estimation of Serum Tri-glycerides, serum cholesterol, Serum LDL, Serum HDL, Serum VLDL, Atherogenic index, Serum glutamate oxalo transaminase, Serum glutamate pyruvate transaminase, Serum alkaline phosphatases,Triton(100mg/kg) induced hyperlipidemia in rats,Estimation of Serum Cholesterol, Serum Tri-glycerides, Serum LDL, Serum HDL, Serum VLDL, Atherogenic index, Serum glutamate oxalo transaminase, Serum glutamate pyruvate transaminase, Serum alkaline phosphate,to investigate in vivo antioxidant effect of ethanolic extract of Ruellia tuberosa roots,Estimation of Lipid peroxidation, Super oxide dismutase, Catalase activity.***

I.INTRODUCTION

Hyperlipidemia

Hyperlipidemia is a disorder characterized by excess lipids in the bloodstream. It is a metabolic dearrangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease.

It is now established that hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. Recent studies have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins. The low-density lipoproteins (LDL) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high-density lipoproteins (HDL) carry cholesterol from peripheral tissues to the liver. HDL thus gives protection against many cardiac problems and obesity.[1]

Causes Of Hyperlipidemia

Mostly hyperlipidemia is caused by lifestyle habits or treatable medical conditions. Lifestyle habits include consumption of fatty food, alcohol and smoking. Medical diseases that may result in hyperlipidemia are diabetes, kidney disease,pregnancy, and hypothyroidism. One can also inherit hyperlipidemia. The cause may be genetic if a patient has a normal body weight and other members of his/her family have hyperlipidemia. One has a greater chance of developing hyperlipidemia in a man older than age 45 or a woman older than age 55. If a close relative had early heart disease, there is also an increased risk of this disease.[2]

Hyperlipidemia in general has no apparent symptoms and it is discovered and diagnosed during routine examination or evaluation for atherosclerotic cardiovascular disease. However, deposits of cholesterol may be formed under the skin in individuals with familial forms of the disorder or in persons with very high levels of cholesterol in the blood.[3]

LIPIDS

The biological molecules that are insoluble in aqueous solutions and soluble in organic solvents are classified as lipids. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building-blocks": ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories.[4]

II.REVIEW OF LITERATURE

Classification Of Hyperlipidemia

Depending on the complexity of the disease, hyperlipidemia is classified into two types.

1) Primary Hyperlipidemia.
2) Secondary / Acquired Hyperlipidemia.

Hyperlipidemia is classified according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. It was later adopted by the World Health Organization (WHO). It does not directly account for HDL, and it does not distinguish among the different genes that may be partially responsible for some of these conditions. In the past it was a popular system of classification but is considered out-dated by many experts now. Following are the five types of hyperlipidemia described by Fredrickson.

Primary Hyperlipidemia

Several genetic conditions are known to responsible for primary Hyperlipidemia, such as lipoprotein lipase deficiency, apolipoprotein C-II deficiency etc... The primary hyperlipidemia may be treated by anti-lipidemic drugs. Primary Hyperlipidemia is again classified into 5 types.

1. Hyperlipoproteinemia Type-I: Severe elevation of CMs with resultant elevation of TGs.
2. Hyperlipoproteinemia Type-IIa: Elevation of LDL only.
3. Hyperlipoproteinemia Type-IIb: Elevation of both LDL and TGs.
4. Hyperlipoproteinemia Type-III: It develops due to defect in VLDL remnant Clearance.
5. Hyperlipoproteinemia Type-IV: It is characterized by hyper TGs.
6. Hyperlipoproteinemia Type-V: It is characterized by elevated levels of CMs and VLDL.

Secondary/Acuired Hyperlipidemia

In this many factors can influence the level of TGs in circulation like diabetes, obesity etc…Secondary Hyperlipidemia demands treatment of original diseases rather than Hyperlipidemia.

Causes Of Secondary Hyperlipidemia

- Metabolic influences: Diabetes, Obesity, Hyperuricemia, Glycogen storage disease type I
- Hormonal influences: Insulin, Estrogen, and Thyroxine.
- Nutritional influences: Alcohol, High carbohydrate intake.
- Disease states: Renal diseases, Renal failure, Nephritic syndrome.
- Drugs: Diuretics, Beta-blockers, Glucocorticoids, Estrogen replacement therapy.[8,9]

Complications Of Hyperlipidemia

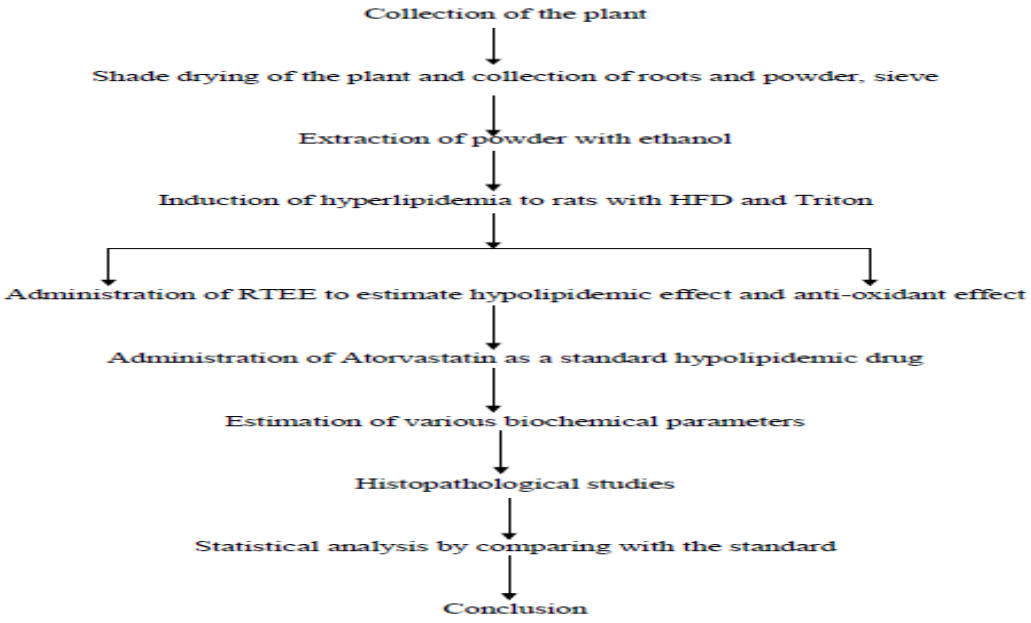
Hyperlipidemia is major risk factor for the atherosclerosis. Other complications are coronary heart disease, ischemic cerebro vascular disease, hypertension, obesity and diabetes mellitus (Type -II).

Plant

Ruellia tuberosa Linn is a low-growing perennial herb with tuberous roots, grown wild and also found in many parts of the world including India. It reaches an average height of about 25cm in moist and shady environments. In India, it is grown in Tirupathi, Talakona forests, Chittoor, Chennai, Punjab and also preferably in grasslands, road sides and waste places.

Figure 2: Ruellia tuberosa plant

Plan of Work



III.MATERIALS AND METHODS

Plant Material

Roots of *Ruellia tuberosa* were collected from the waste places and grass lands around Madanapalle, Chittoor district, ANDHRA PRADESH, INDIA. The plant was identified and authenticated by Dr. S. VIJAYAKUMAR, professor, Department of Pharmacognosy, Sri krishna chaithanya college of pharmacy, Madanapalle, Chittoor district, Andhra Pradesh, India.

Preparation of Plant Extract

The collected plant was washed thoroughly with water and dried in the shade.

The shade dried plant was taken and roots were separated from the plant and powdered, sieved using sieve number40.1kg of the obtained powder was extracted with 95% ethanol by Soxhlet extraction method. After completion of the extraction the solvent was removed by rotary evaporator method. The ethanolic extract was used for further study. The yield obtained from the above process was found to be 15.3 % w/w and the extract was brownish yellow. The extracts were preserved in a refrigerator.[10]

Preliminary Phytochemical Screening

The different qualitative tests were performed for establishing profile of the given extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present in them.

Detection of alkaloids.

- **Mayer’s reagent**
- **Wagner's reagent**
- **Dragendroff's reagent**

Detection of carbohydrates

- **Molisch's test**
- **Fehiling’s / Barfoed’s / Benedict,s test**

Detection of glycosides

- **Millon's test**
- **Biuret test**
- **Ninhydrin test**

Detection of phytosterols

- **Libermann- Buchard's test**

Detection of phenolic compounds and tannins

- **Ferric chloride test**
- **Lead acetate test**
- **Alkaline reagent test**

Methodology

Experimental Animals

Albino rats (130±20gms) were procured from Mahaveer Enterprises, Hyderabad, India and used for the experiment. Rats were maintained in an air-conditioned room (25±2°C) with a normal night and day cycle. Rats were fed with standard pellet diet and demineralized drinking water ad libitum. The rats were allowed to acclimatize to the laboratory environment for a week before the start of the experiment. All experimental procedures were conducted in conformity with Animal Ethics committee (Reg. No. Number SKCP/IAEC/PGCOL/15-16/04) for the care and use of animals and were strictly followed throughout the study.

Experimental Design

Acute Toxicity Studies[11]

Acute toxicity study for the ethanol extract of Ruellia tuberosa roots was done according to the OECD guidelines No: 423 and dose was selected for treatment.

Method

Depending on the mortality and or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance.

Evalauation of Antioxidant Activity[12]

Estimation of Lipid peroxidation assay

Lipid peroxidation in the homogenate was determined by measuring the amounts of malondialdehyde produced primarily. 0.2 ml of tissue homogenate, 0.2 ml of 8.1 % of sodium dodecyl sulphate, 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 absorbance was read at 532 nm against an appropriate blank without the sample. The values were expressed as nm of MDA formed /mg of protein values are normalized to protein content of tissues.

Estimation of super oxide dismutase

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 absorbance was measured at 480nm. SOD was expressed as unit/mg protein.

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 H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as unit/mg protein.

Triton Induced Hyperlipidemia[13]

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological

Collection of blood

On the 11thday, 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. The liver sections were isolated and preserved in 10% formalin. The liver sections were evaluated for histopathology to assess any architectural changes.

Biochemical analysis

The serum extract was assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), Serum glutamate oxalo transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Serum alkaline phosphatase using standard protocol methods.

III. RESULTS

Phytochemical Screening

Ethanol extract of *RTEE* was subjected to preliminary phytochemical analysis to test for presence or absence of

various phytochemical constituents and the following results obtained.

CHEMICAL CONSTITUENT	RTEE
Alkaloid	-ve
Glycoside	-ve
Saponins	-ve
Carbohydrates	-ve
Tannins	+ve
Flavanoids	+ve
Amino acids	-ve
Phenols,Triglycerides	+ve

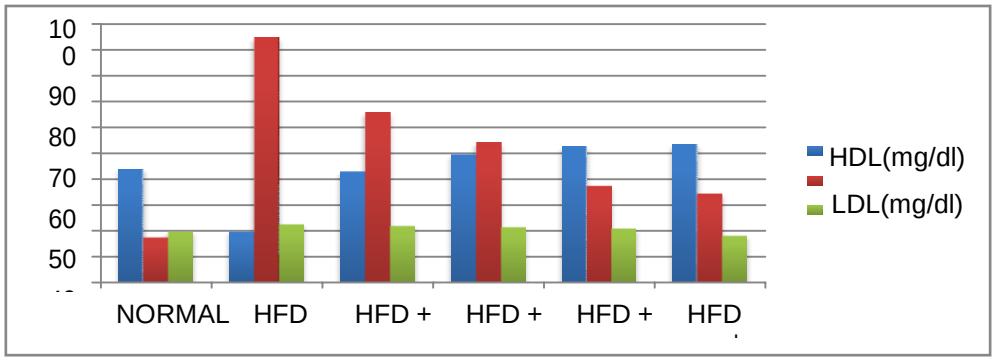
Table : Phytochemical screening of RTEE

High Fat Diet Induced Hyperlipidemia In Rats

Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg, p.o., once daily) on serum lipid Parameter levels in rats fed with HFD for 30 days.

S.NO	GROUP	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDL : HDL
1	NORMAL	43.88± 1.79	17.42±1.61	19.62±0.42	0.404
2	HFD	19.59±1.59	94.63±1.16	22.48±0.35	4.83
3	HFD+ RTEE DOSE I	42.81±3.91	65.67±3.88	21.83±1.48	1.534
4	HFD+ RTEE DOSE II	49.30±2.97	54.12±1.54	21.25±0.63	1.097
5	HFD+ RTEE DOSE III	52.60±0.78***	37.14±1.23**	20.8±0.51***	0.706
6	HFD+ STD	53.38±0.92***	34.15±1.44***	17.95±0.37***	0.639

Table 10: LDL- Low density lipoprotein, HDL- High density lipoprotein, and VLDL- Very low density lipoprotein. n = 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01,



P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test. Comparison of RTEE 2012

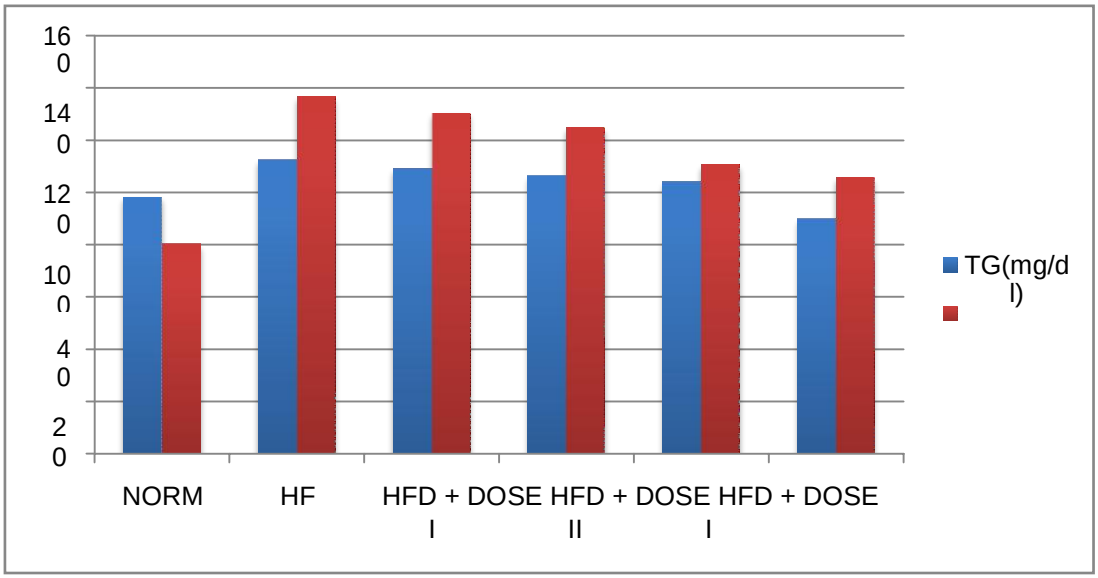
with Control group and of Control group with Normal group.

Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg, p.o., once daily) on TC, TG levels in rats fed with HFD for 30 days.

S.NO	GROUP	TG (mg/dl)	TC (mg/dl)	ATHEROGENIC INDEX
1	NORMAL	98.12 ± 3.72	80.12 ± 1.48	0.82
2	HFD	112.4 ±1.59	136.7 ±1.76	5.98
3	HFD+ RTEE DOSE I	109.16 ±1.45	130.3 ±2.82	1.54
4	HFD+ RTEE DOSE II	106.28 ±2.62	124.67±2.38	1.21
5	HFD+ RTEE DOSE III	104.02 ±0.60***	110.54±1.24**	0.98
6	HFD+ STD	89.75 ±0.37***	105.48±2.16***	0.68

Table 11: TC-Total Cholesterol, TG-Triglycerides. n=6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01,P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test Comparison of RTEE with Control group and of Control.

Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg,p.o., once daily) on SGOT, SGPT, ALKALINE PHOSPHATASE levels in rats fed with HFD for 30 days.

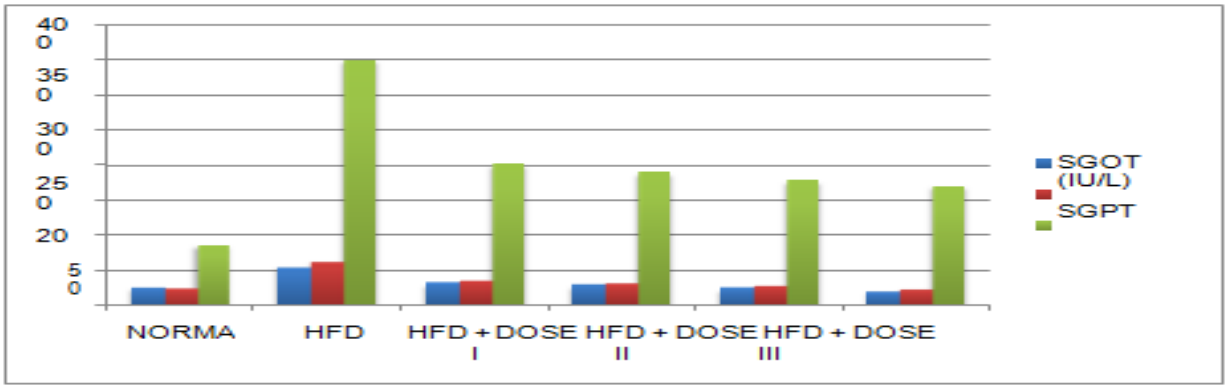


S.NO	GROUP	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
1	NORMAL	25.65 +0.83	24.65 + 1.71	86.45 +1.13
2	HFD	54.75 ±0.61***	62.57 ±3.40***	350.66 ±1.54***

3	HFD+ RTEE DOSE I	31.56 ±1.96**	33.45 ±0.66**	201.41 ±1.31**
4	HFD + RTEE DOSE II	28.25 ±1.70**	29.55 ±0.80**	189.62 ±0.84***
5	HFD + RTEE DOSE III	24.14 ±1.47**	25.93 ±0.79***	178.08 ±1.14***
6	HFD + STD	20.34 ±1.38***	22.61 ±0.96**	170.81 ±0.51***

Table12: SGOT- Serum glutamate oxalo transaminase, Serum glutamate pyruvate transaminase. Serum alkaline phosphatase. n = 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01, P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test. Comparison of RTEE with Control group and of Control group with Normal group.

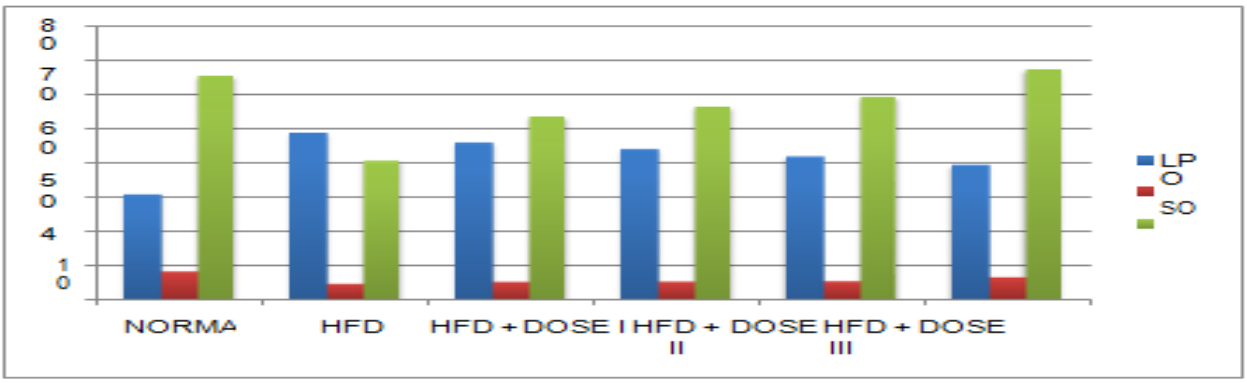
Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg, p.o., once daily) on LPO, SOD, CAT levels in rats fed with HFD for 30 days.



S.NO	GROUP	LPO	SOD units/mg	CAT units/mg
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 13: LPO- Lipid peroxidation, CAT- Catalase, SOD- Superoxide dismutase. n =6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01, P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test Comparison of RTEE with Control group and of Control group with Normal group.

FIG12:: **LPO- Lipid peroxidation, CAT- Catalase, SOD- Superoxide dismutase. n = 6 animals in each group**

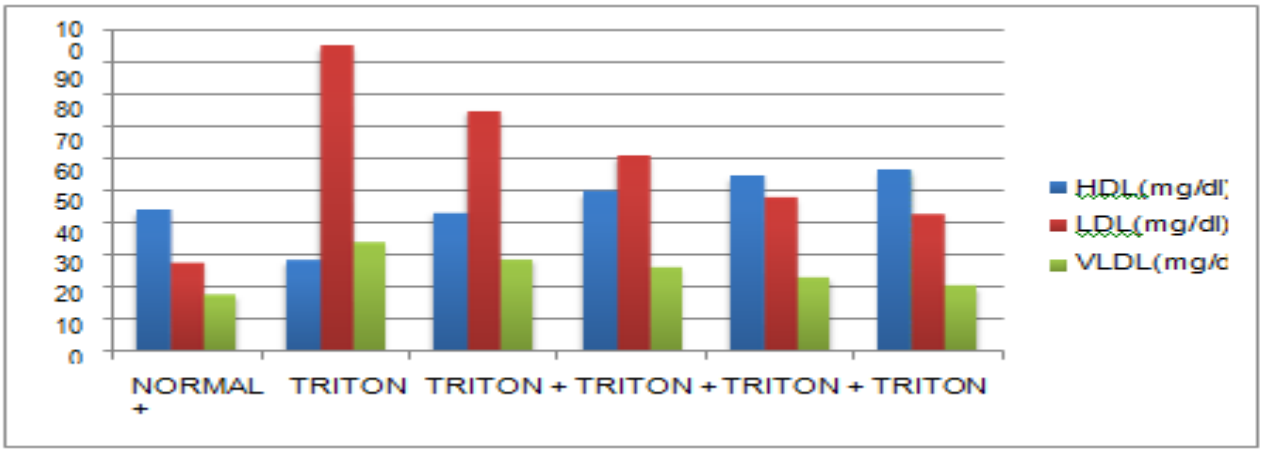


TRITON INDUCED HYPERLIPIDEMIA IN RATS:

7.3.1: Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg,p.o., once daily) on serum lipid Parameter levels in rats fed with Triton for 10 days.

S.NO	GROUPPP	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDL: HDL
1	NORMAL	43.87±0.55	27.35±2.36	17.49±0.75	0.62
2	TRITON TREATED	28.31±3.33	94.78±1.82	33.74±0.87	3.34
3	TRITON+ RTEE DOSE I	42.9±0.30	74.79±1.04	28.48±0.74	1.74
4	TRITON+ RTEE DOSE II	49.59±0.86	60.79±0.88	26.12±0.24	1.22
5	TRITON+ RTEE DOSE III	54.38±0.80***	47.64±1.06***	22.86±0.24***	0.87
6	TRITON+STD	56.27±1.45***	42.46±0.63***	20.39±1.02***	0.75

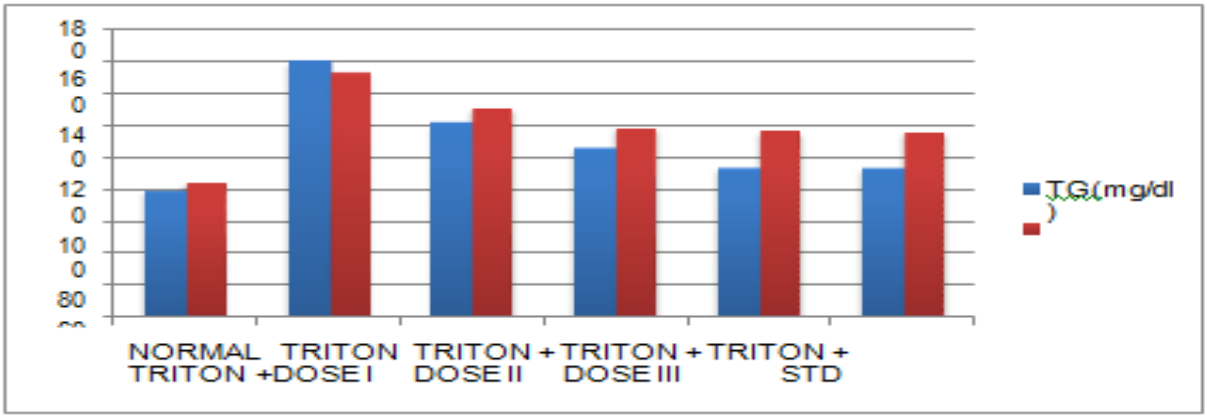
Table 14: TC – Total Cholesterol, TG – Triglycerides, LDL – Low density lipoprotein, HDL – High density lipoprotein, VLDL – Very low density lipoprotein. n= 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01, P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test. Comparison of RTEE with Control group and of Control group with Normal group.



5: Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg, p.o., once daily) on TG, TC levels in rats fed with Triton for 10 days.

S.NO	GROUP	TG (mg/dl)	TC (mg/dl)	ATHEROGENIC INDEX
1	NORMAL	78.96±1.46	84.28±0.86	0.92
2	TRITON TREATED	160.24±1.15	152.58±0.89	4.35
3	TRITON+ RTEE DOSE I	121.53±1.75	130.46±1.19	2.04
4	TRITON + RTEE DOSE II	106.49±0.94	118.68±1.07	1.76
5	TRITON+ RTEE DOSE III	93.01±1.13***	116.24±1.72**	1.13
6	TRITON+ STD	92.98±1.19***	115.27±1.71***	1.04

Table 15:TC – Total Cholesterol, TG – Triglycerides. n = 6 animals in each group. Values are expressed as mean ± SEM. Statistically significant at P**≤0.01, P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test. Comparison of RTEE with Control group and of Control group with Normal group.



6: Effect of administration of RTEE (100/250/500mg/kg, p.o., once daily)/Atorvastatin (10mg/kg, p.o., once daily) on TG, TC levels in rats fed with Triton for 10 days.

S.NO	GROUP	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
1	NORMAL	58.96 + 2.35	53.78 + 1.54	101.2 + 1.32
2	TRITON	87.85 ± 2.78	91.31±1.91	309.43 ± 3.62
3	TRITON + RTEE DOSE I	80.25 ± 2.19	65.08 ± 1.74	198.01 ± 4.42

4	TRITON + RTEE DOSE II	52.62 ± 3.94	60.11±3.59	183.06 ± 2.29
5	TRITON + RTEE DOSE III	47.85±1.45	51.24±2.35	179.10± 1.92
6	TRITON + STD	45.32 ± 2.53	52.32±2.52	179.31 ± 2.24

Table16: SGOT- Serum glutamate oxalo transaminase, SGPT- Serum glutamate pyruvate transaminase. ALP - Serum alkaline phosphatases. n = 6 animals in each group. Values are expressed as mean ± SEM Statistically significant at P**≤0.01, P*** ≤ 0.001.Variation analysis done by One way ANOVA followed by Dunnet's test Comparison of RTEE with Control group and of Control group with Normal group.

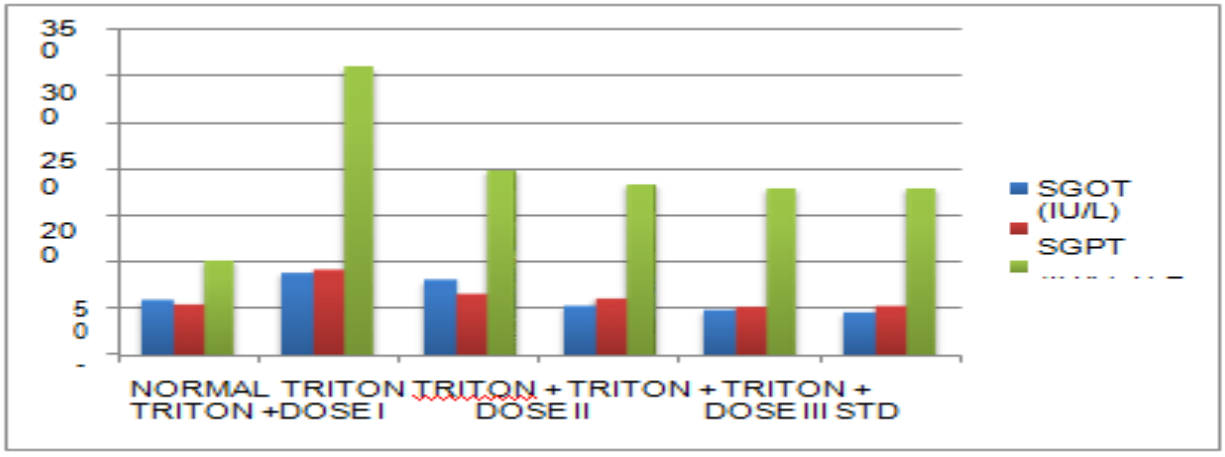


FIG7:**SGOT- Serum glutamate oxalo transaminase, SGPT- Serum glutamate pyruvate transaminase. ALP - Serum alkaline phosphatases. n = 6 animals in each group**

HISTOPATHOLOGICAL STUDIES

Histopathology of liver of different groups:

Slide 1: Histological examination of the normal liver slices showed normal hepatic fattychanges with a sign of inflammation and necrosis in rats.

Slide 2: The liver tissue of HFD and TRITON (hyperlipidemia) treated rats showed fatty changes (micro vesicular steosis).

Slide 3: Liver tissue of hyperlipidemia induced rats treated with 100 mg/kg of RTEE showed reduction in fatty material with little inflammation and necrosis.

Slide 4: Liver tissue of hyperlipidemia induced rats treated with 250mg/kg of RTEE shows nearly normal reduction in fatty material and inflammation and necrosis.

Slide 5: : Liver tissue of hyperlipidemia induced rats treated with 500mg/kg of RT EE shows very much reduction in fatty material and no sign of inflammation and necrosis.

Slide 6: The liver tissue of hyperlipidemia induced rats treated with Atorvastatin and ethanolic extract of *RTEE* at 500 mg/kg dose showed reduction of fatty material to a great extent.

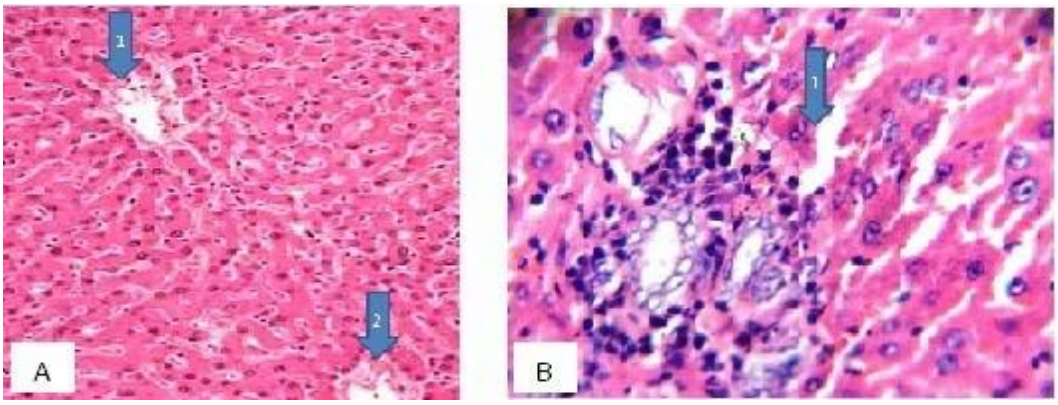


FIG:9 Photomicrograph of the liver tissue Photomicrograph of the liver tissue of HFD and of normal control group TRITON induced hyperlipidemia(fatty changes)

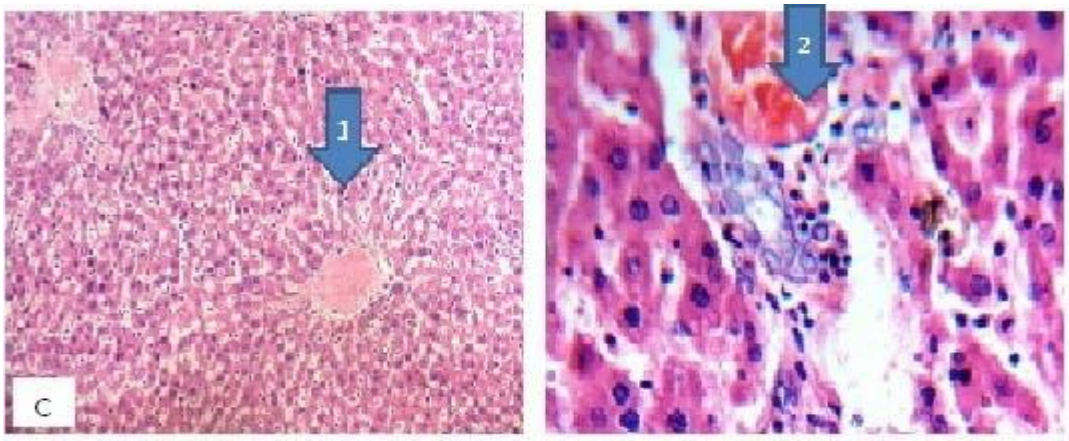


FIG10 : Photomicrograph of the liver tissue treated with RTEE(100 mg/kg) showed mild reduction in fatty material with little necrosis.

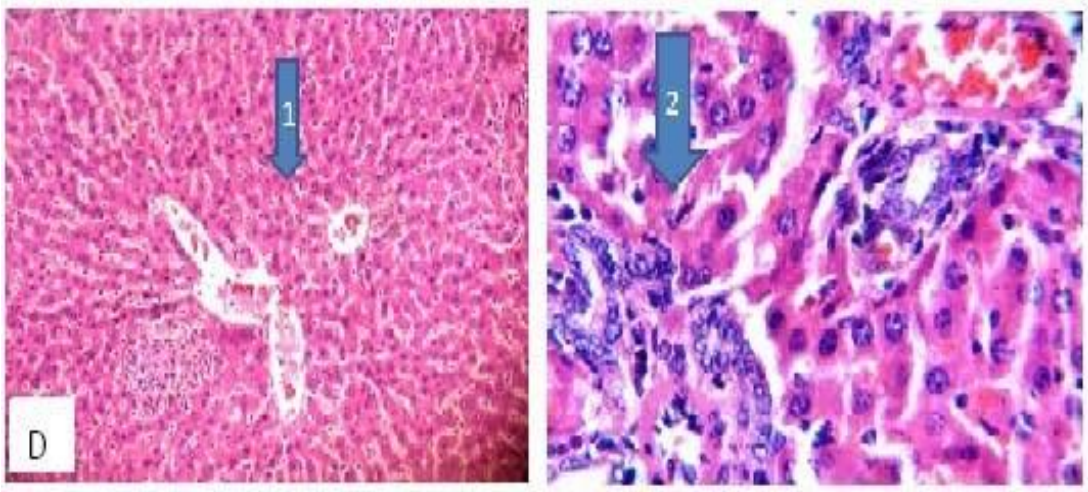


FIG11: Photomicrograph of the liver tissue treated with RTEE(250 mg/kg) showed nearly great reduction in fatty material.(no necrosis.)

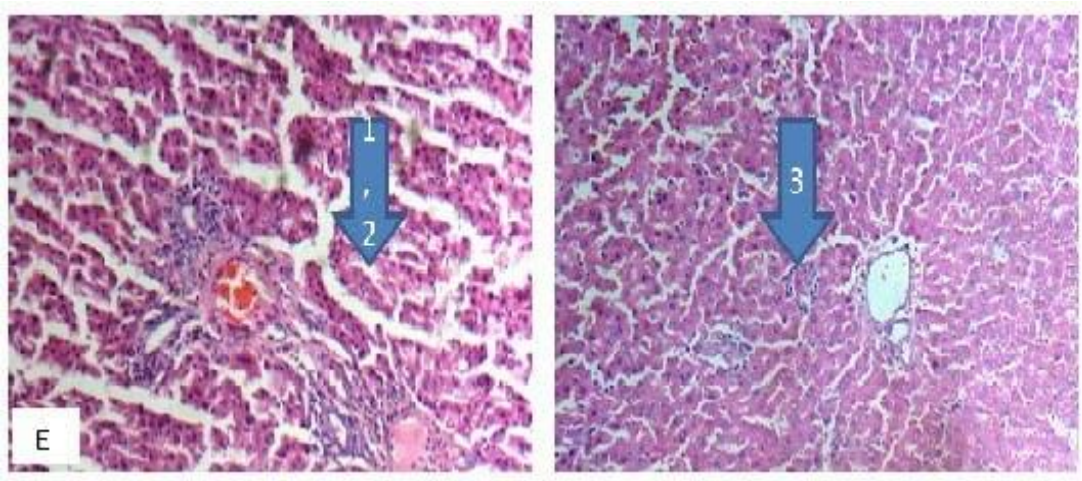


FIG12: Photomicrograph of the liver tissue treated with RTEE(500 mg/kg) showed very great rreduction in fatty material.(no necrosis.)

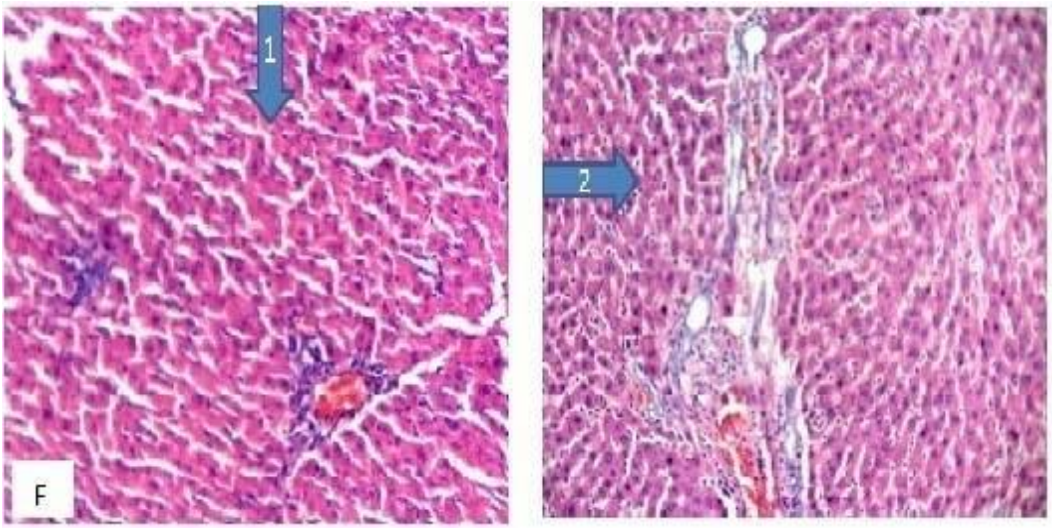


FIG13: Photomicrograph of the liver tissue treated with ATORVASTATIN (10 mg/kg) showed very great reduction in fatty material

IV.DISCUSSION

The above results show that RTEE indicates the presence of tannins, flavonoids, triglycerides and phenols.Hence the total lipids i.e. total cholesterol and triglycerides in plasma as well as LDL and VLDL cholesterol were significantly reduced at three doses of feed supplementation. However, HDL cholesterol level increased in drug treated groups significantly. This observation indicates that, as a feed component is effective in reducing serum LDL and VLDL levels. It is well known that increased HDL levels have a protective role.

The results also shows that SGOT, SGPT, Alkaline phosphatase in serum was significantly reduced at three doses of feed supplementation. However, HDL cholesterol level increased in drug treated groups significantly. This observation indicates that, as a feed component is effective in reducing serum SGOT, SGPT, Alkaline phosphatase levels.

The results shows that the RTEE 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 oxidation. The results of our study showed that administration of high fat diet induced significant production of MDA in liver, and administration of RTEE significantly decreases the MDA production in liver. RTEE also resulted in a significant increase in the liver CAT, SOD as compared to the control animals, which suggests its antioxidant activity.

Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. 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. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack. 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. The antioxidant enzymes, mainly superoxide dismutase and catalase are first-line defensive enzymes against free radicals. The qualitative analysis of RTEE 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. The currently noted elevated levels of SOD and catalase with RTEE could be due to the influence of tannins, flavonoids,triglycerides and phenols.

In triton induced hyperlipidemic model, the groups treated with the RTEE 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. The groups treated with the RTEE and Atorvastatin demonstrated significant decrease in the Atherogenic Index and LDL: HDL risk ratios. 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.

The groups treated with the RTEE 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. These figures illustrate the protective action of the RTEE against fatty infiltration and granular degeneration due to hyperlipidemia closely comparable to that with Atorvastatin. The RTEE showed a significant antihyperlipidemic activity in the animal model and the best activity was shown by RTEE.

V.SUMMARY AND CONCLUSION

In folk medicine, ***Ruellia tuberosa*** **L.** has been used as anti-diabetic, antipyretic, analgesic, anti hypertensive, thirst quenching, antidotal agent and this plant 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. Roots were used to treat kidney diseases; syrup of root was used to treat whooping cough; infusion or decoction for a diabetes remedy. 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.

Chronic hyperlipidemia was induced by feeding female rats HFD (cholesterol-25mg/kg) for 30 days.Acute hyperlipidemia was induced by administration of Triton (100mg/kg, i.p., at once) in female rats for 10 days.Administration of RTEE (100/250/500mg/kg) for 30 days in HFD model and RTEE (100/250/500mg/kg) for 10 days in triton models respectively, successfully prevented the elevation of serum TG, TC, LDL, VLDL, SGOT, SGPT, Alkaline Phosphatase and decrease of serum HDL in HFD and Triton model rats in a dose dependent manner.Treatment with RTEE for 30 days successfully prevented the elevated liver homogenate LPO levels indicating its efficacy to reduce the LDL-c oxidation and decreases of in-vivo antioxidant enzyme catalase, superoxide dismutase.Hypolipidemic activity was observed with 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.In conclusion, the findings of the study suggest that RTEE is a potent anti hyper cholesterol emic drug lowering LDL, VLDL and increasing HDL levels in two hyperlipidemic models such as HFD, Triton. The mechanism has point towards inhibiting cholesterol and triglyceride synthesis.The drug also demonstrated for antioxidant properties in in-vivo antioxidant models.

REFERENCED.

1. H. Ryan; Diet and exercise in the prevention of diabetes; Int. J. Clin. Pract; 134, 2003, 28-35.
2. G.Davey Smith; Cholesterol lowering and mortality: the importance of considering initial level of risk; Int .Med. J, 306(6889), 1993, 1367-1373, Correction: 1648.
3. T. M.Speight and Avery's; Drug treatment Principles and Practice of clinical Pharmacology and therapeutics; Third Edition, ADIS press Ltd, 1987, 599.
4. Klein C, Malviya AN; Mechanism of nuclear calcium signaling by inositol 1,4,5- trisphosphate produced in the nucleus, nuclear located protein kinase C and cyclic AMP- dependent protein kinase; Frontiers in Bioscience, 13 (13), 2008, 1206– 26.
5. Vasu Keshetty et.al, Antihyperlipidemic activity of methanolic extract of garlic (Allium sativum L.) in Triton X-100 induced hyperlipidemic rats; J of Pharma Res, 2(5), 2009, 777-780.
6. Sapakal V. D. et.al, A review on in-vivo screening of antioxidant profile, Journal of Herbal Medicine and Toxicology, 2 (2), 2008, 1-8
7. Jude E, Okokon, Bassey S, Antia and Emem umoh; Analgesic and Anti inflammatory effects of Ethanolic Root extract of Hippocratea Africana; International J of Pharmacology, 4(1), 2008, 51-55.
8. Sharpless KB, Snyder TE, Spencer TT, Maheswari KK, Guhn G, and Clayton RB. J Am Chem Soc 1969, 91(13), 3394.
9. Vinay Kumar, Abul K Abbas and Nelson F. Pathological basis of disease, seventh Edition. New Delhi, Elsevier, 2002.
10. Khandelwal KR. Practical Pharmacognosy Techniques and experiments. Nirali Prakashan. 2007; 149-156
11. Gehard Vogel H. Drug discovery and evaluation-Pharmacological assays. Second edition, Berlin: Spinger-Verlag; 2001, 1095-1125.
12. Jude E, Okokon, Bassey S, Antia and Emem umoh; Analgesic and Anti inflammatory effects of Ethanolic Root extract of Hippocratea Africana; International J of Pharmacology, 4(1), 2008, 51-55.
13. Vasu Keshetty et.al, Antihyperlipidemic activity of methanolic extract of garlic (Allium sativum L.) in Triton X-100 induced hyperlipidemic rats; J of Pharma Res, 2(5), 2009, 777-780.