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Evaluation of hypolipidemic activity of ethanolic extract of *Trema orientalis* L. Blume leaves

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Abstract
It is traditionally used for its various properties, and hence in the present study, alcoholic extract of *Trema orientalis* L. Blume (EETO) leaves has been screened for its hypolipidemic activity. Hypolipidemic activity is screened by inducing hyperlipidemia with the help of Fructose induced rat model, Triton x 100 induced model and serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol were determined. The atherogenic index shows the measure of the athero-genic potential of the drugs. The alcoholic extract showed significant ($p < 0.05$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as a significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Simvastatin. The alcoholic extract exhibited significant atherogenic index and percentage protection against hyperlipidemia. The EETO possesses significant antioxidant activity evidenced by elevated levels of glutathione reductase and Glutathione peroxidase compared to the hyperlipidemic group. The ratio of HMG-CoA to mevalonate is an index of enzyme activity which catalyses the conversion of HMG to mevalonate. These biochemical observations were, in turn, confirmed by histopathological examinations of liver sections and are comparable with the standard hypolipidemic drug Simvastatin. The overall experimental results suggest that the biologically active phytoconstituents such as flavonoids, glycosides alkaloids present in the alcoholic extract of *Trema orientalis* L. Blume (EETO). The leaves may be responsible for the significant hypolipidemic activity, and the results justify the use of *Trema orientalis* L. Blume as a significant hypolipidemic agent.

Keywords: *Trema orientalis*, hypolipidemic, ethanolic extract

Introduction
According to World Health Organization (WHO), in 2008, 17.3 million people died from cardiovascular diseases (CVDs); of these deaths, 7.3 million were due to coronary heart disease, and 6.2 million were due to strokes. CVDs are projected to remain the single leading cause of death, representing 30% of all global deaths. The number of people, who died from CVDs, mainly from heart disease and stroke, will be expected to reach 23.3 million by 2030.
Modern drugs of the first choice for elevated LDL, cholesterol are not free from side effects, particularly when used for prolonged periods. They are associated with adverse effects like myopathy, gastrointestinal disturbance, rashes, and adverse psychiatric reactions include depression, memory loss, and confusion. (Fedacko *et al.*, 2010) [3]. As none among the available agents fulfil requirements of the desired drug, there is a need to explore the possibility of introducing effective, safe and inexpensive alternatives.
Plant-derived foods and beverages are rich in phenolic compounds that show protection properties against cancer, cardiovascular disease and ageing (Hollman and Katan, 1999) [4]. LDL oxidation (oxLDL) is considered to be a significant risk factor for the development of atherosclerosis and CVD (Witztum, 1994) [13]. Both human and animal *in vivo* studies have shown that the level at which LDL oxidises, decreases linearly with increasing phenolic concentration (Covas *et al*., 2006; Weinbrenner *et al*., 2004; Marrugat *et al*., 2004) [2, 14, 5].
Thus, attention is now directed to the natural products from plant origin that possess the antiatherosclerotic activity and can promote human health. This can eventually avoid possible health effects due to the long period consumption of statins.
According to the literature review, antihyperlipidemic activity on *Trema orientalis (L.) Blume* has not been investigated scientifically so far. Keeping because of the therapeutic efficacy of herbal medicines, the plant**.** *Trema orientalis (L.) Blume* has been selected to evaluate antihyperlipidemic activity.

2. *In vivo* studies
2.1 Selections of animals
Twenty four male *Wistar albino* rats weighing between 150-170 gm. were obtained from Small Animal Breeding House, Mannoothy, Thrissur, Kerala and housed at Animal house of

Department of Pharmaceutical Sciences, MGU, RIMSR, Rubber board P.O., Kottayam, The animals were housed in polypropylene cages in a room where the congenial temperature 27±1°C, 30-60 % relative humidity and 12 hours light and dark cycles were maintained.

The animals were allowed to acclimatise to the environment for 14 days and randomly divided into five groups. They were fed with standard pellet diet collected from Hindustan Lever Limited, Bangalore and water were given ad libitum. The experiments were carried out after obtaining the permission of Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences MGU, RIMSR, Rubber board P.O., Kottayam, Kerala, India under IAEC No: DPS/16/2015

The antihyperlipidemic activity of EETO was assessed in both chronic and acute models. The biochemical parameters and histopathological analysis were carried out to assess the effectiveness of EETO on hyperlipidemic conditions. The methods are described as follows: -

2.2 Fructose induced rat model

Hyperlipidemia was induced in rats by feeding 25% fructose for 30 days. From days 15 to 30 days. Trema *orientalis* L. Blume alcoholic extract (250mg/kg, 500mg/kg) was administered p.o. The results were compared with the group of animals treated with simvastatin and control groups.

Wistar albino male rats weighing 150-200 gm were selected and divided into five groups. There were six animals in each group. All experiments were carried out round the clock.

Group I: Control group receives 2ml of 0.5%w/v of CMC orally.

Group II: Hyperlipidemic group in which the rats were daily feeding fructose at a dose of 25% solution (25gm in 100ml water)

Group III: Feed fructose as in group II and Simvastatin at a dose of 10mg/kg body weight from days 15 to 30.

Group IV: Feed fructose as in group II and Trema *orientalis L. Blume* alcoholic extract at a dose of 250mg/kg body weight from days 15 to 30.

Group V: Feed fructose as in group II and *Trema orientalis L. Blume* alcoholic extract at a dose of 500 mg/kg body weight from days 15 to 30.

Trema orientalis L. Blume alcoholic leaf extract and Simvastatin were fed to the rats using a rat feeding tube. A homogenous suspension of the extract and standard drug Simvastatin was freshly prepared by using 0.5% w/v of carboxy methyl cellulose. Rats were fed daily with the standard diet.

The normal control group was treated with the vehicle instead of drugs. Blood samples were collected from all experimental animals on day 31th by retro-orbital puncture method, and subsequently, serum was separated for analysis of serum lipid profile parameters. After collection of the blood samples, the animals were sacrificed by cervical decapitation from each animal; the liver was excised and stored at 80^{0}C until subsequent analysis of the antioxidant activity of hepatic tissue samples.

The main biochemical parameters recommended by National Cholesterol Education Program guidelines (2002) for lipid screening like total cholesterol (T.C.), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol(HDL-C) and triglycerides (T.G.) were evaluated from serum. The triglycerides (T.G.), total cholesterol (T.C.) and HDL cholesterol were measured using assay kits (Spin diagnostics). Low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) were calculated using Friedewald's formula. (LDL=TC-HDL-TG/5.0mg/dL)

2.3 Triton x 100 induced model (acute model)

Usharani *et al*., 2014.

Twenty-four male *Wistar albino* rats were randomly divided into five groups of 6 each. The first group was given standard pellet diet, water and orally administered with 0.5% CMC. The II, III, IV, V group animals were injected 10% aqueous solution of Triton X 100 by i.p at a dose of 400mg/kg body weight.

After 72 hrs of triton injection, the second group received a daily dose of 0.5% CMC p.o for seven days. The third group was administered with standard drug Simvastatin 10mg/kg p.o for seven days. The fourth and fifth group was administered a daily dose of *Trema orientalis L.* Blume extract, 250mg/kg and 500 mg/kg suspended in 0.5% CMC p.o for seven days after inducing hyperlipidemia. Food was withdrawn 10 hrs before blood sampling.

Group I: Normal group in which the rats were administered with 0.5% CMC orally.

Group II: Hyperlipidemic group in which the rats were administered with Triton X 100 at a dose of 400 mg/kg body weight.

Group III: Standard group in which the rats were administered with Simvastatin at a dose of 10 mg/kg body weight after 72 hrs. of Triton X 100 injection.

Group IV: Test group in which the rats were administered with. *Trema orientalis* L. Blume extract, at a dose of 250mg/kg after 72 hrs. of Triton X 100 injection.

Group V: Test group in which the rats were administered with *Trema orientalis* L. Blume extract, at a dose of 500mg/kg after 72 hrs. of Triton X 100 injection.

On the 8th day, blood was collected by retro-orbital sinus puncture under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analysed for serum Total Cholesterol, Triglycerides, High-Density Lipoprotein Cholesterol, Low-Density Lipoprotein Cholesterol and Very Density Lipoprotein Cholesterol.

Triglycerides, Cholesterol, HDL-cholesterol were measured with enzymatic kits. The LDL, VLDL–Cholesterol, was calculated by Friedewald's formula. (LDL=TC-HDL-TG/5.0mg/dL)

2.4 Histopathological Analysis

The liver samples were collected under anaesthetised (ether) conditions, and a portion of the liver was fixed in 10% formalin for a week at room temperature. The specimens were dehydrated from 70% ethanol up to 100% ethanol in a graded series, then cleared in xylene and embedded with paraffin wax in moulds.

The specimens were then sectioned into 5 µm thick using a rotary microtome. Sections were stained with hematoxylin and eosin dye, and photomicrographs were obtained under a light microscope. The sections were observed for histoarchitecture alterations like mononuclear cell infiltration within the lobules and necrotic damage to hepatocyte structures.

3. Biochemical estimations in liver homogenate

3.1 Preparation of Hepatic Tissue Samples for Analysis

Hepatic tissue (100mg tissue/mL buffer) was first homogenised in 50mM phosphate buffer (pH7.2); the homogenate was then centrifuged at 12,000rpm for 15mins, and the supernatant was used for analysis.

3.2 Estimation of HMG-CoA reductase activity (HMG CoA/Mevalonate Ratio)

The ratio of HMG-CoA to mevalonate was taken as an index of enzyme activity, which catalyses the conversion of HMG to mevalonate. The lower the ratio higher the enzyme activity. The liver sample (100mg) was homogenized in 1.0mL of arsenate (1gm/L) solution. Equal volumes (0.5mL each) of fresh 10% liver tissue homogenate and diluted perchloric acid(50 mL/L) were mixed.

This was allowed to stand for 5 minutes and centrifuged at about 2000rpm for 10min. This was filtered, and 1mL of the filtrate was mixed with 0.5mL of freshly prepared hydroxyl amine (2mol/L) reagent of pH 5.5 for HMG-CoA and with 0.5mL of freshly prepared hydroxyl amine (2mol/L) reagent of pH 2.1 for mevalonate.

After 5 minutes, 1.5mL of freshly prepared ferric chloride reagent (prepared by dissolving 5.2 gm of trichloroacetic acid and 10 gm of ferric chloride in 50mL of 0.65mol/L HCl was added to each of the test tubes for HMG-CoA and mevalonate. The tubes were shaken well. Absorbance was read after 10 min at 540nm versus a similarly treated arsenate blank. (Rao *et al*., 1975)

3.3 Estimation of glutathione reductase

Glutathione reductase catalyses the reduction of oxidised Glutathione to reduced Glutathione. This enzyme, which is found in many tissues enables the cell to sustain adequate levels of cellular GSH. Reduced Glutathione is a substrate for the glutathione peroxidases, which provide a mechanism for the detoxification of peroxidases, and glutathione S-transferases, which are involved in the conjugation and elimination of xenobiotics from the organism.

Reduced Glutathione also acts as an antioxidant reacting with free radicals and organic peroxidases. About 50µl of the sample was taken along with a 3ml reaction mixture containing sodium phosphate buffer (0.1M, pH 7.4), 0.5mM EDTA, 1mM GSH and 1mM NADPH. The mixture was then read at 340nm using spectrophotometer**.** (Venkateswaran *et al*., 2014) [10].

3.4 Estimation of glutathione peroxidase

The enzyme glutathione peroxidase catalyses the oxidation of reduced GSH to oxidised form, which reacts with NADPH and gets converted to the oxidised form of NADP and two molecules of reduced Glutathione, which is measured spectrophotometrically at 340nm**.** (Venkateswaran *et al*., 2014) [10].

4. Statistical Analysis

Graph pad Prism Version 6.0 was used for statistical analysis. Results were expressed as mean ± SEM Data was analysed by one-way ANOVA followed by Dunnett comparisons test. The P values < 0.05 were considered as statistically significant.

5. Results

5.1 Triton Induced Hyperlipidemia: Acute Model

The results obtained from the biochemical estimation of serum lipid profiles of the animals belonging to various treatment groups of Triton X 100 induced hyperlipidemia model activity were expressed as Mean ± SEM. The total cholesterol, HDL-C, LDL.-C, VLDL-C, and T.G.s were assessed and tabulated in table no. 1.

In Triton X 100 induced model, oral administration of ethanolic extract of leaves of *Trema orientalis* 250 mg,500 mg/kg significantly reduced total serum cholesterol (T.C.), triglyceride (T.G.), low density lipoprotein-cholesterol (LDL-C), VLDL and increase HDL compared with the positive control group.

Table 1: Estimation of biochemical parameters of EETO on Triton induced model (Acute model)

Model	Treatment Groups	Total Cholesterol (Mg/Dl)	Triglyceride Level (Mg/Dl)	Hdl Level (Mg/Dl)	Ldl Level (Mg/Dl)	Vldl Level (Mg/Dl)
Triton Induced Model	Control	89.33 ± 1.585***	97.67 ± 0.578***	37.83 ± 0.791***	39.83±0.732***	14.50 ± 0.67***
	Hyperlipidemic Group	162.0 ± 2.380	136.5 ± 2.680	16.83 ± 0.9458	79.67 ± 2.765	30.17 ± 0.70
	Standard - Simvastatin 10 Mg/Kg	97.17 ± 1.579***	92.83 ± 1.138***	31.83 ± 1.352***	46.33 ± 2.29***	17.33± 0.667***
	Eeto [500MG/Kg]	126.0 ± 2.436**	117.5 ± 2.604**	20.67 ± 0.802**	60.17 ± 2.25**	21.50 ± 0.76**
	Eeto [250MG/Kg]	136.7± 1.961*	122.7 ± 2.290*	19.83±0.609(ns)	66.17 ± 1.64*	24.83 ± 1.01*

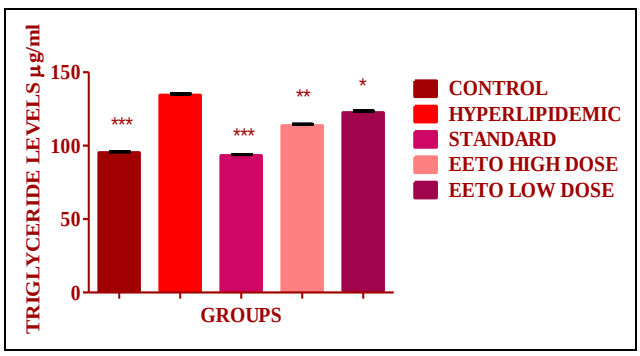


Fig 1: Effect of EETO on triglyceride level

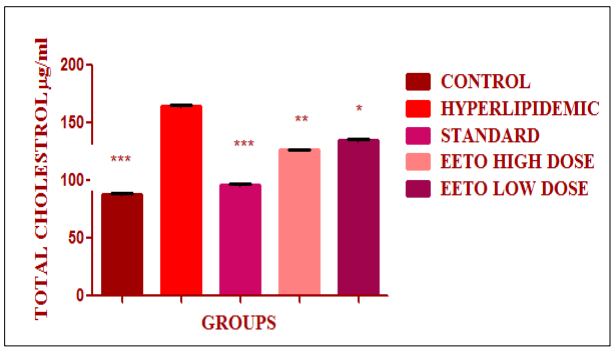


Fig 2: Effect of EETO on total cholesterol level

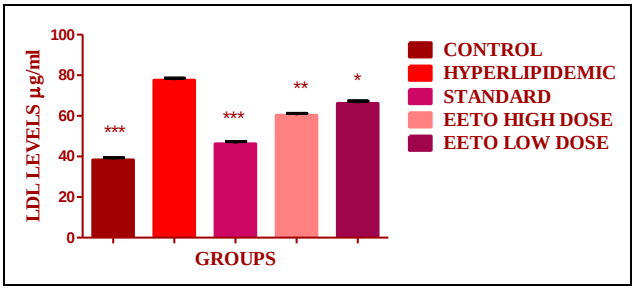


Fig 3: Effect of EETO on LDL level

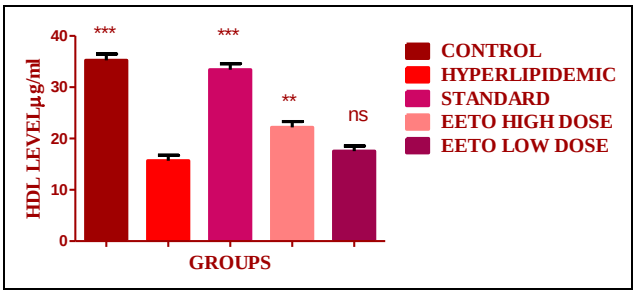


Fig 4: Effect of EETO on HDL level

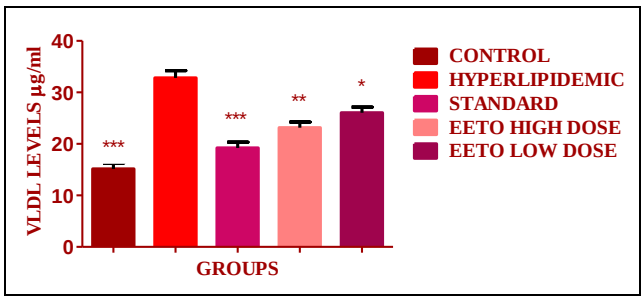


Fig 5: Effect of EETO on VLDL level

This study showed that the serum lipid parameters in animals were significantly reduced after (p<0.001) seven days treatment with EETO at dose levels 250 mg/kg and 500 mg/kg when compared with the positive control group. 500 mg/kg EETO group animals have shown more significant (p<0.001) compared with the positive control group

Values are statistically evaluated by One way ANOVA followed by Dunnet's Multiple Comparison Test. *Represents statistical significance vs. positive control, * P<0.05, *** P<0.001 as compared to the positive control.

5.2 Fructose induced hyperlipidemia

The results obtained from the biochemical estimation of serum lipid profiles of the animals belonging to various treatment groups of Fructose induced hyperlipidemia model. The results were expressed as Mean ± SEM. The total cholesterol, HDL-C, LDL.-C, VLDL-C, and T.G.s were assessed. The EETO at the dose of250 and 500mg/kg p.o significantly reduced total serum cholesterol (T.C.), triglyceride (T.G.), low density lipoprotein-cholesterol (LDL-C), VLDL cholesterol levels but significantly increased serum HDL-cholesterol level as compared with the positive control group in Fructose induced animal model.

Table 2: Estimation of biochemical parameters.

Model	Treatment Groups	Total Cholesterol (Mg/Dl)	Triglyceride Level (Mg/Dl)	Hdl Level(Mg/Dl)	Ldl Level (Mg/Dl)	Vldl Level (Mg/Dl)
Fructose Induced Model	Control	85.83 ± 0.3073***	98.67 ± 0.3333***	38.13 ± 0.7491***	38.73 ± 0.7032***	13.15 ± 0.68***
	Hyperlipidemic Group	165.8 ± 3.081	139.0 ± 2.781	18.17 ± 0.7491	81.50 ± 3.354	29.67 ± 0.499
	Standard - Simvastatin 10 Mg/Kg	92.33 ± 2.390***	90.83 ± 1.579***	31.50 ± 1.478***	45.83 ± 2.75***	18.12 ± 0.67***
	Eeto [500mg/Kg]	119.5 ± 2.895***	115.0 ± 2.221***	27.17 ± 1.276***	61.47 ± 2.12***	22.33 ± 0.71***
	Eeto [250mg/Kg]	130.2 ± 1.447***	120.2 ± 1.922***	22.33 ± 0.9545***	67.00 ± 1.87***	25.17 ± 0.702**

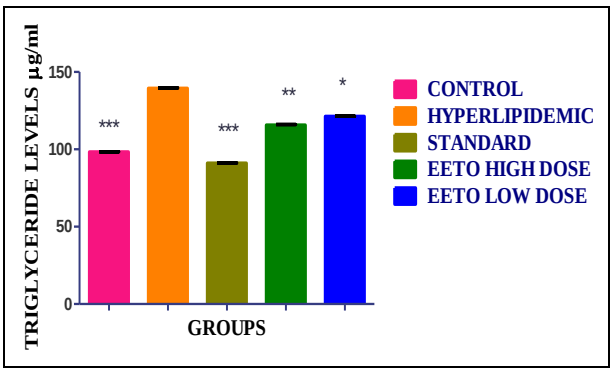


Fig 6: Effect of EETO on Triglyceride

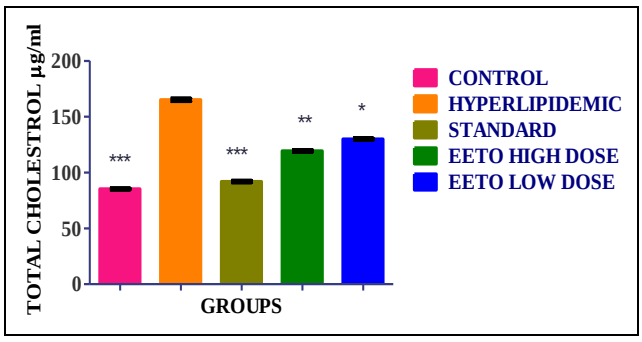


Fig 7: Effect of EETO on Total Cholesterol

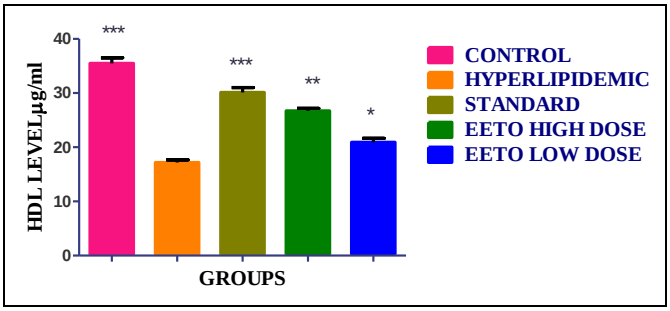


Fig 9: Effect of EETO on HDL

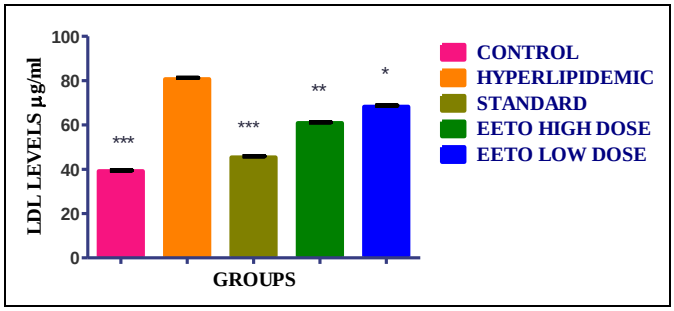


Fig 8: Effect of EETO on LDL

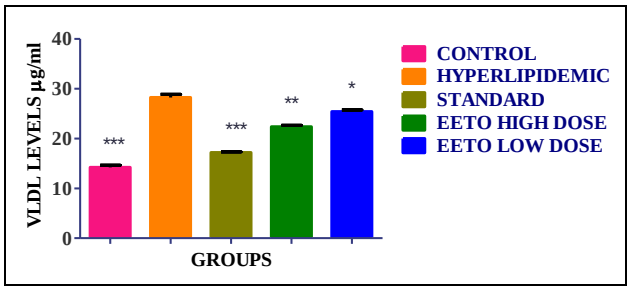
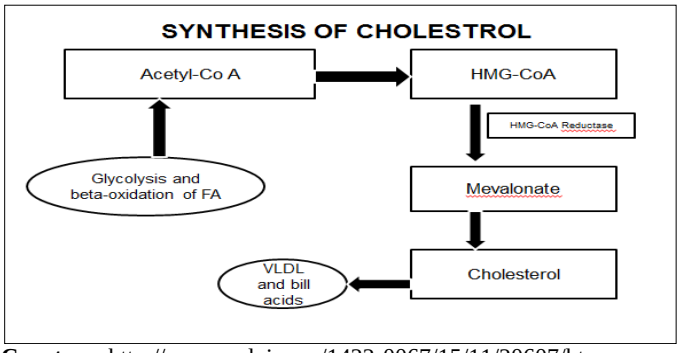


Fig 9: Effect of EETO on VLDL level

This study shows that lipid parameters in animals were significantly reduced (p<0.001) by fifteen days after treatment with EETO at the dose levels 250 mg/kg and 500 mg/kg when compared with positive control Simvastatin 10mg/kg. EETO at the dose of 500mg /kg animal has shown very significantly (p<0.001) compared with the positive control group.

Values are statistically evaluated by One way ANOVA followed by Dunnet's Multiple Comparison Test. *Represents statistical significance vs. positive control, * P<0.05, *** P<0.001 as compared to the positive control.

5.3 Possible mechanism of EETO



Courtesy: http://www.mdpi.com/1422-0067/15/11/20607/htm

Fig 10: Possible Mechanism of EETO

Glycolysis and beta-oxidation of fatty acid produce acetyl- Co A, and then it converted to mevalonate. This conversion may be inhibited by EETO lead to inhibition of cholesterol synthesis. So it may help to reduce the total cholesterol, triglyceride, LDL and VLD and HDL may be increased.

6. *Ex Vivo* Results

6.1 Effect of Eeto on Liver Hmg /Mevalonate Ratio, Glutathione Reductase and Glutathione Peroxidase Levels Of Hyperlipidemic Rats.

Table 3: Effects On Fructose Induced Hyperlipidemic Model. (Chronic Model)

S. No.	Treatment (P.O)	Hmg/Mevalonate Ratio	Glutathione Reductase Level (Units/Ml)	Glutathione Peroxidase Level (Units/Ml)
1	Control	2.58 ± 0.24**	0.512 ± 0.14***	1.03 ± 0.12***
2	Hyperlipidemic	0.34 ± 0.12	0.105 ± 0.20	0.06 ± 0.10
3	STANDARD (Simvastatin10mg/K)	3.25 ± 0.29***	0.498 ± 0.21***	0.601 ± 0.12**
4	EETO (500mg/kg)	2.81 ± 0.24**	0.365 ± 0.12**	0.311 ± 0.26*
5	EETO(250 mg/kg)	2.39 ± 0.11*	0.201 ± 0.47*	0.239 ± 0.10*

Values are in MEAN±SEM; data were analysed by one -way ANOVA followed by Dunnet's multiple comparison test. *p< 0.05, **p<0.01, ***p<0.001, as compared to positive control. From the above table No: 8.9, it was clear that EETO possesses significant antioxidant activity evidenced by elevated levels of glutathione reductase and Glutathione peroxidase compared to the hyperlipidemic group. The ratio of HMG-CoA to mevalonate is an index of enzyme activity which catalyses the conversion of HMG to mevalonate. Lower the ratio higher the activity and vice versa. HMG -CoA reductase activity was measured in the liver homogenate indirectly and was found to be increased like that of standard drug Simvastatin

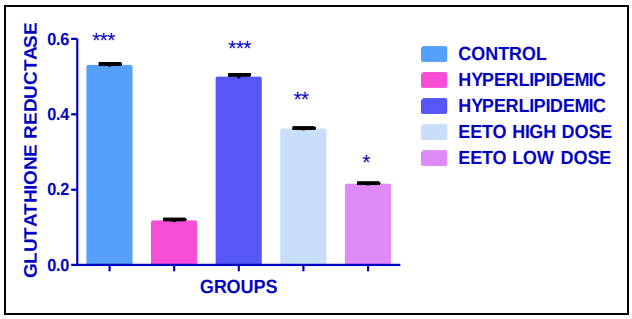


Fig 11: Effect of EETO on HMG/Mevalonate ratio

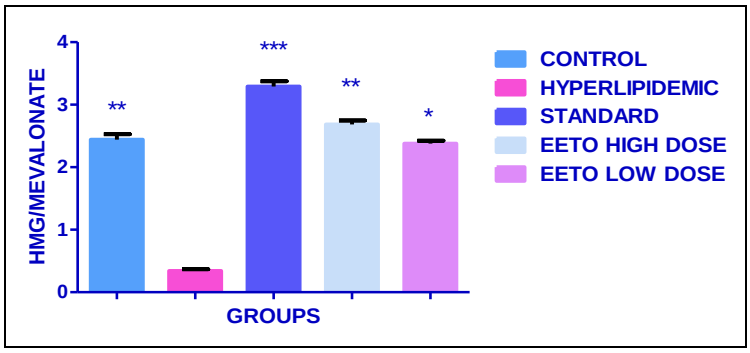


Fig 12: Effect of EETO on Glutathione Reductase level

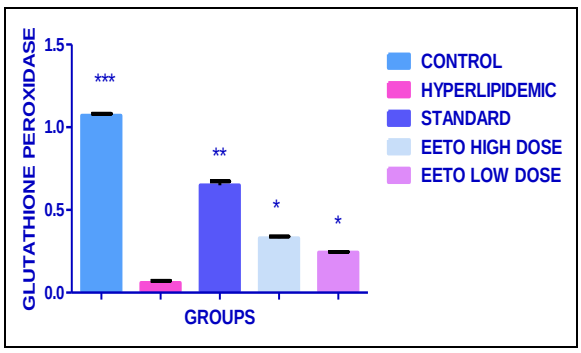
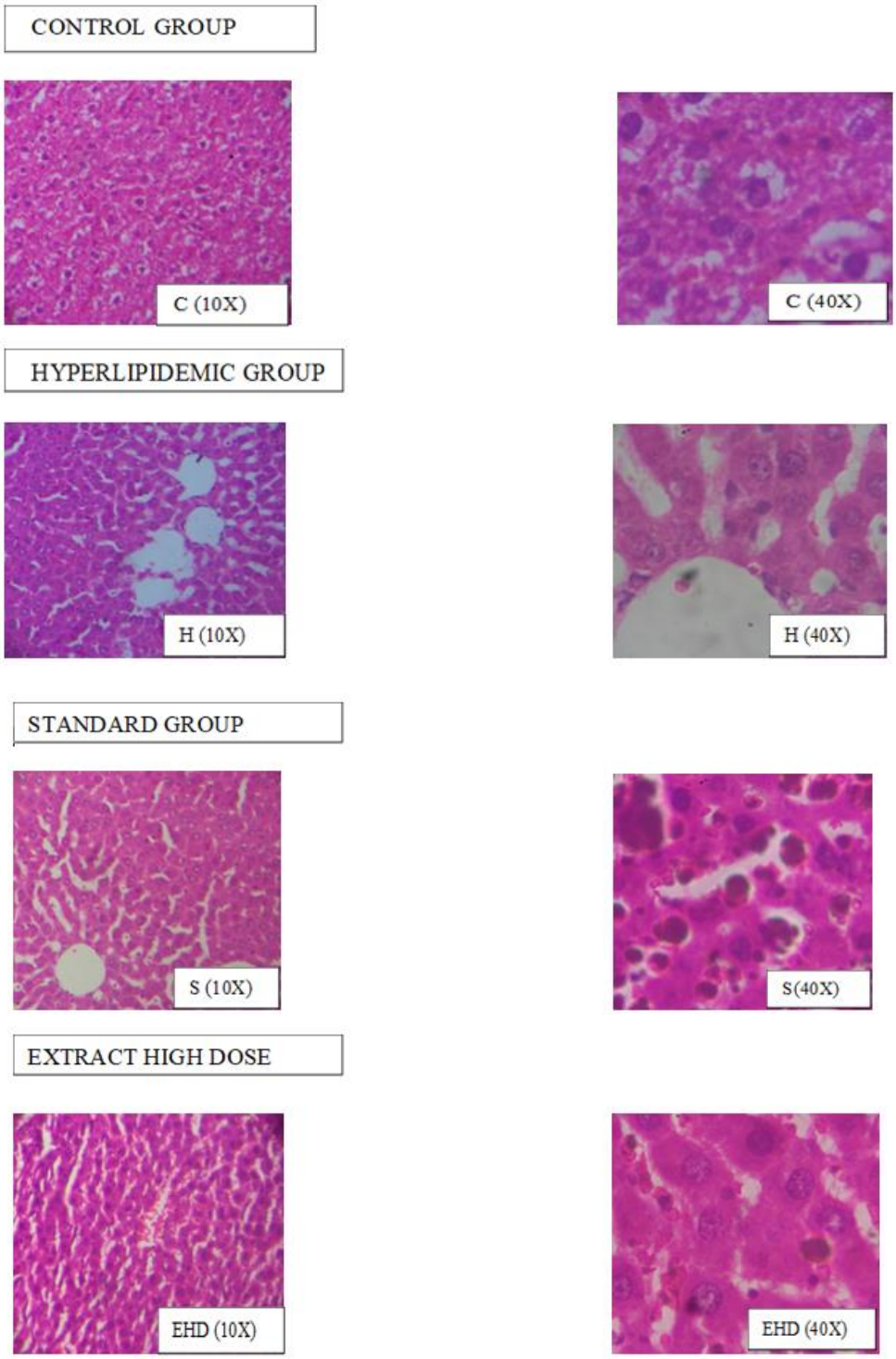


Fig 13: Effect of EETO on Glutathione Peroxidase level

7. Histopathological Analysis

Histoarchitecture of *Wistar rats* after treatment with 25% Fructose, standard (Simvastatin 10 mg/kg) and EETO at a dose of (250,500 mg/kg) are obtained as follows;



Sections of hepatic tissue from the experimental groups of rats were stained by H&E and then subjected to histopathological examination by light microscopy.

Control group: Sections of hepatic tissue from control rats showed that central vein with normal hepatocyte, healthy nucleus, and sinusoidal spaces with kpuffer cells.

Hyperlipidemia group: Sections from hypercholesterolemic rats revealed that loss of normal liver radiating pattern, periportal inflammation with cellular infiltration in a central vein.

Standard: Sections from standard treated rats, there is mild steatosis.

Extract high dose: Sections from EETO (500mg/kg) showed mild steatosis. The peripheral inflammatory changes are noticeable but not much significant. Sinusoidal dilatation is very mild.

Extract low dose: Sections from EETO treated group (250mg/kg) showed moderate grade steatosis but comparatively lesser than the hypercholesterolemia group. Mild periportal inflammatory changes also present.
Hypercholesterolemia induced hepatic abnormalities confirmed by histopathological findings. Treatment with EETO appeared to ameliorate or prevent the adverse effects, as suggested by the presence of only minimal or partial fatty changes.

8. Discussion
We evaluated the antihyperlipidemic activity of EETO, both in acute and chronic experimental models. Acute hyperlipidemia was induced by administering Triton X- 100. Triton, a surfactant blocks the clearance of triglyceride-rich lipoprotein from circulation by extrahepatic tissues, resulting in increased circulatory lipids.
Triton X-100 induced rise in serum triglyceride is possibly due to hypoactivity of lipoprotein lipase in blood vessels, which breaks up triglyceride. The high T.G. level, along with decreased absorption of fatty acids by adipose, is associated with a low level of HDL-C, insulin resistance and increased risk of atherosclerosis.
Administration of EETO for seven days caused a significant decrease in serum T.C., LDL-C, VLDL-C and an increase in HDL, suggesting a beneficial modulatory influence on cholesterol metabolism and turn over. The hypolipidemic activity of EETO may be due to increased removal of lipoproteins and triglycerides from the circulation.
The chronic hyperlipidemia was induced by administering a 25% fructose solution to the animals for 30 days. Fructose induced hyperlipidemia in rats is an established animal model to evaluate antihyperlipidemic drugs. Chronic hyperlipidemia finally alters morphological and haemodynamic functions of the heart as well as the liver.
In the present study, EETO caused significant hypolipidemic activity in Fructose induced model. Fructose administration to rats caused a significant elevation in serum T.C., T.G., VLDL-C, and LDL-C and the concomitant decrease in HDL-C. Treatment of Fructose fed rats with EETO (250mg/kg and 500mg/kg), and Simvastatin (10 mg/kg) caused a significant reduction in the elevated serum T.C., T.G., VLDL-C, and LDL-C, and restored the decreased HDL in serum.
The lipid-lowering effect of EETO in rats may be due to the inhibition of cholesterol biosynthesis and increased faecal bile acid excretion (Patil *et al.*, 2004) [6].
LCAT plays a key role in incorporating free cholesterol into HDL and transferring back to VLDL or IDL, which is taken back by the liver cells (Raj Lakshmi and Sharma, 2004) [7]. HDL functions as "good cholesterol"; it resists atherosclerosis directly, by transferring cholesterol and triglycerides from the peripheral tissues to the liver where it is catabolised and excreted out of the body through hepatic receptors (Wilson *et al.*, 1988) [12]. In our study on hyperlipidemic rats, administration of EETO significantly increased the HDL-C levels. This may be due to the enhancement of lecithin cholesterol acyltransferase (LCAT) and the inhibition of hepatic. Triglyceride lipase (HTL) on HDL-C, which may lead to rapid catabolism of blood lipids through extrahepatic tissues. (Anila and Vijayalakshmi, 2002) [1].
Triglycerides play a vital role in the regulation of lipoprotein interactions to maintain normal lipid metabolism. Increased serum T.G. levels were associated with an amplified rate of coronary artery disease. The observed serum hypotriglyceridemic effect on the administration of EETO could be due to the enhanced catabolism of triglyceride. As hypothesised by many workers with other plants (Sudheesh *et al.*, 1997) [9], the restoration of the catabolic metabolism of triglycerides could be due to increased stimulation of the lipolytic activity of plasma lipoprotein lipase (LPL).In the present study, HMG CoA reductase activity was indirectly measured in terms of the ratio of HMG CoA and mevalonate. The ratio is inversely proportional to HMG CoA reductase activity. In this study, EETO treatment inhibited HMG CoA reductase dose dependently.
The formation of highly reactive free radicals during hyperlipidemia causes oxidative stress, which ultimately leads to atherogenesis. The reduced GSH is one of the major non-enzymatic antioxidant presents in the liver and other tissues. In cholesterol fed rats, GSH will be reduced due to increased utilisation of GSH to augment the activities of antioxidant

enzymes, GPx, SOD and catalase. (Shaw *et al.*, 1993) [8]. The restoration of depleted GSH and GPx were observed in both in EETO and Simvastatin treated groups. It indicated that EETO could preserve the activity of the antioxidant enzyme in high-lipid organs, rectify the metabolic disturbance of free radicals, maintain the dynamic balance of the oxidation and antioxidation systems, reduce the toxic side-effects of free radicals, and reduce the damage of lipid peroxidation.

Histological examination reveals that steatosis, periportal inflammation, and sinusoidal dilatation caused by cholesterol administration are not much significant or mild in EETO treated groups. The above mentioned pharmacological activities could probably due to phytoconstituents like steroids, triterpenoids, and phenolics present in the leaf extract.

Thus, the present study revealed hypolipidemic activity of ethanolic extract of leaves of *Trema orientalis* L. Blume

9. Conclusion

Findings of the *in-vivo* studies revealed that EETO decreases the elevated serum lipid level in a dose-dependent manner both in acute and chronic models of hyperlipidemia.

In light of our pharmacological studies, it can assume that the hypolipidemic activity of EETO may be due to the presence of phytoconstituents, especially steroids, phenolics and flavonoids. The present study has given some preliminary idea about the antihyperlipidemic compounds present in the ethanolic extract of *Trema orientalis* (L.) Blume.

10. References

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