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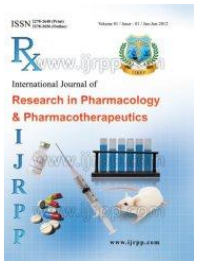

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Evaluation of anti-hyperlipidemic activity of *clerodendrum serratum linn on* high cholesterol diet induced hyperlipidemia in rats

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ABSTRACT

The main objective of the study was to investigate about the antihyperlipidemic activity of the ethanolic extract from the aerial parts of *Clerodendrum serratum Linn* (EECS). Qualitative analysis of the extract revealed that it contains glycosides, alkaloids, tannins, proteins, amino acids, phytosterol, steriods, terpenoids & saponins. Acute oral toxicity study was performed. Animals were divided into five groups. Normal control, positive control, Std-I-Rosuvastatin + 5% cholesterol diet, Extract of EECS-250 mg + 5% cholesterol diet, Extract of EECS- 500mg + 5% cholesterol diet for 12 weeks. Parameters considered for evaluation Weight gain ,Blood samples were collected, parameters such as serum lipid profile (TC, TG, HDL-c, LDL-c, VLDL-c), Kidney parameters (Urea, uric acid, Creatinine) and antioxidant enzymes such as (SOD, GSH, Catalase) were analysed. Findings suggested that *Clerodendrum Serratum* Linn. is a potent drug for hyperlipidemia, the drug extract treated groups lowers TC, TG, LDL-c & VLDL-c and increases the levels of HDL-c and antioxidant enzymes such as SOP, GSH and Catalase in all high cholesterol treated groups. Kidney parameters such as urea, creatinine and uric acid levels were decreased in all EECS treated group.

Keywords: *Clerodendrum serratum*, Hyperlipidemia, Antioxidant, Cholesterol

INTRODUCTION

Obesity has reached epidemic proportions globally, with more than 1 billion adults overweight - at least 300 million of them clinically obese - and is a major contributor to the global burden of chronic disease and disability. Often coexisting in developing countries with under-nutrition, obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups[1]. Development of obesity is, however, more complicated than that; sedentary life style, genetic factors, medical illness, microbiological aspects, social factors and neurobiological mechanisms are also involved[2-3].

Several of these problems contribute to the earlier mortality associated with obesity and include coronary artery disease, severe hypertension that may be refractory to medical management, impaired cardiac function, adult-onset (type 2) diabetes mellitus, obesity hypoventilation and sleep apnea syndromes, cirrhosis, venous stasis and hypercoagulability with an increased risk of pulmonary embolism, and necrotizing panniculitis. Extreme obesity has been estimated to truncate the lifespan of young adults by 5–20 years.[4] The medical problems caused by obesity begin at the head and end at the toes and involve almost every organ in between. Ayurvedic system of medicine is one of the oldest system of medicine having a history of more than 3000 years. Several prototype derived from these herbal medicines are in use for various kind of disease and disorders. It not only gives new molecule but also with newer mechanism of action, hence is called Gold mine. Several infusions or decoctions of plants used in traditional medicine to reduce obesity could be utilized to delete the clinical side effects of the current chemically formulated antiobesity agents; examples include *Camellia sinensis* (L.) Kuntze (Theaceae), *Chlorella pyrenoidosa* Chick. (Oocystaceae), *Citrus aurantium* L. (Rutaceae), *Garcinia cambogia* L. (Clusiaceae), *Lagerstroemia speciosa* (L.) Pers. (Lythraceaea). For the current research we have taken *Clerodendrum serratum linn.* to evaluate its anti-obesity. As per survey from the literature it was found that flavonoids, sitosterols, tannins and saponins have shown the anti-obesity activity by various mechanisms. Clerodendrum serratum have shown the presence of some common phytoconstituents which is useful in the treatment of obesity and was selected for phytochemical analysis, screening its anti-obesity to substantiate the traditional claim.

Plant Name: *Clerodendrum Serratum* Linn, Family: Verbenaceae, Kingdom: Plantae, Order: Lamiales, Genus: *Clerodendrum*, Species: Serratum. Vernacular Names are Tamil: chirudekku, Bharangi, Angaravalli, Hindi: Bharangi, Malayalam: Brahmanayshtika. The plant *Clerodendrum serratum* is widely distributed all over India and especially in the Himalaya region, Tamilnadu, Madhya Pradesh, peninsular region etc.[5]. A slightly woody shrub with bluntly quadrangular stems and branches, leaves usually three at a node, sometimes opposite oblong or elliptic, serrate; flowers blue, many in long cylindrical thyrsus; fruits 4 lobed purple durpe, somewhat succulent with one pyrene in each lobe. It is used for treatment of conditions as asthma, cough, fever, etc. Plant pacifies vitiated kapha, pitta, inflammations, dyspepsia, flatulence, helminthiasis, cough, asthma, bronchitis, hiccough, chronic skin diseases, leucoderma, leprosy and fevers. The leaves can be used or external application in headache. As per the traditional claims roots are the potential source of drugs for aliments such as, body ache, bronchitis, cholera dropsy, eye disease, inflammation, malaria, snake bite, rheumatism, tuberculosis wounds and ulcer.[6-7]

MATERIALS AND METHODS

Collection & authentication of plant

The aerial parts of *Clerodendrum serratum Linn.* were collected from surrounding areas of Komarapalayam town, Namakkal District, Tamilnadu, India. The plant specimen was authenticated by Prof. Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy research Center, Chennai., Tamilnadu, India. Reg No: PARC /2014/3041.

Extraction procedure

The aerial parts of *Clerodendrum Serratum* Linn were dried under shade, mixed together, and then made in to a coarse powder with a mechanical grinder. The powder was stored in an airtight container for further use. The dried powder material (500gm) was extracted with ethanol (70%v/v) in a Soxhlet extractor for 72 hr. The solvent was then distilled off and the resulting semisolid mass was dried in a vacuum evaporator to get a yield of 12.8 %w/w.

Preliminary phytochemical analysis

The extract of *Clerodendrum Serratum* Linn was subjected to qualitative tests for the identification of various plant constituents like alkaloids, glycosides, tannins, saponins, proteins and amino acids, phytosterol and steroids,and terpenoids.[8-10]

PHARMACOLOGICAL STUDIES

PROCEDURE: GROUPS

Group I : Control (Normal control)
Group II : Positive control (5% cholesterol diet for 3 months)
Group III : Standard(Rosuvastatin (20mg/kg) + 5% cholesterol diet)
Group IV : Test I (Plant Extract-250mg/kg B.W of EECS+ 5% cholesterol diet)
Group V : Test II (Plant Extract-500mg/kg B.W of EECS+5% cholesterol diet)

EECS at dose of 250 and 500 mg/kg B.W, through oral route. Throughout the study period Group III-V receives 5% cholesterol diet for last 28 days. After the study period animals were sacrificed by collecting blood through cardiac puncture and collected blood was centrifuged and serum was collected and biochemical analysis were performed lipid profiles for serum levels TC, TG, HDL, LDL, VLDL, Urea, Creatinine, uric acid and antioxidant studies SOD, GSH and Catalase.

RESULTS AND DISCUSSION

Hyperlipidemia means presence of a high amount of cholesterol in the body has been demonstrated to elevate total cholesterol and may increase the risk of atherosclerosis and lead to cardiovascular disease. Herbs play a key role in the management of various CVD. Numerous medicinal plants and their formulations are used for hyperlipidemia in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for hyperlipidemia; most of the herbal drugs speed up the reduction of cholesterol by healthy dietary intake. So, the search for anti-hyperlipidemic activity of high cholesterol induced rats. The preliminary phytochemical screening revealed the presence of Carbohydrates, Alkaloids, Glycosides, Flavonoids, Tannins, Phenolic compounds, Amino acid, Saponins, Steroids, Gums and Proteins.[12] The extract was found safe up to a dose of 5000mg/kg body weight. The dried extract was suspended in 1% CMC at dose levels 250mg/kg and 500mg/kg body weight for oral administration. Cholesterol powder was purchased and used with normal diet shows changes in serum cholesterol levels differ significantly between rats and humans. To clarify these confusions, a 12 week feeding study with rats was conducted to evaluate the influence of high fat diet on serum lipid profiles, kidney parameters and antioxidant studies. The use of rats as experimental animals for hyperlipidemic activity is mainly due to the synthesis and structural similarities in human and rats. The present study carried out inducing 5% cholesterol diet for the experimental induction in rats. Cholesterol will deposits in the endothelial cells and transport with lipoproteins in blood stream which leads to increase in the TC, TG, LDL, VLDL levels and decreases the HDL levels in the body. It is synthesised in the liver and converted into bile acids and excreted through faeces and due to hyper cholestremia urine failure will occur due to oxidative stress.

Rosuvastatin is a competitive inhibitor of HMG-CoA reductase and catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA)
to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases hepatic cholesterol, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes and decrease the amount of LDL-cholesterol in the blood. Like other statins, Rosuvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.[13] The assessment of cholesterol function can be made by estimating the body weight and activities of various lipid profiles such as TC, TG, HDL, LDL and VLDL; kidney parameters such as urea, creatinine and uric acid. Body weight can be increased due to the high cholesterol diet induced in hyperlipidemia rats. As the dietary fat, FFAs can be synthesised into many tissues by transport of increased lipoproteins in the blood stream absorbed from the intestine and metabolised in liver. The EECS treatment and Rosuvastatin drug significantly lower the body weight by the reduction in the LDL, VLDL levels and increase HDL levels.TC and TG are present in dietary fat, FFAs combine with glycerol to form TG and cholesterol is esterified by ACAT to form cholesterol esters, storing cholesterol in cells.[14] TC and TG are generated by the liver and circulate as chylomicrons interact at capillaries of adipose tissues and muscle cells. LPL hydrolyzes the

TG, and FFAs are released. The elevated TC and TG levels in hyperlipidemia induced high cholesterol diet are due to the increase of cholesterol in capillary cells. The EECS treatment and Rosuvastatin drug significantly lowered the abnormal levels of TC and TG might be due to lowering of FFA (Free fatty acids) synthesis in the plasma. LDL and VLDL are package of TG and TC esters in liver and released into blood circulation. VLDL is then hydrolyzed by LPL in tissues to release fatty acids taken up by muscle cells for energy and glycerol becomes a VLDL remnant taken up by the liver by LDLR. They hydrolyzed in the liver by HL to form LDL. LDLR activity and uptake regulates plasma LDL concentration through decreasing the synthesis of HMG-CoA reductase controls the rate of de novo cholesterol synthesis by the cell[14]. LDL and VLDL levels are increased due to the suppression of synthesis of new LDLR in the cells, activates the enzyme ACAT, free cholesterol into cholesterol ester by cholesterol diet. The EECS and Rosuvastatin treatment significantly decrease the LDL and VLDL levels in the liver. HDL is a key lipoprotein involved in reverse cholesterol transport and transfer of cholesterol esters between lipoproteins and secreted by the liver and intestine proceed through a series of conversions known as the HDL cycle. It attract cholesterol from cell membranes and free cholesterol to the core of the HDL particle, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidize LDL[15]. Flavonoids can increase HDL-C and also decreases oxidation of LDL- cholesterol. The EECS and Rosuvastatin treatment significantly increase the HDL levels due to the synthesis of the liver enzymes by converting bile into bile acids and excreted through faeces by decreasing TC and TG levels. Urea, uric acid and creatinine levels were increase due to the high cholesterol diet.[16] There is a association between hypercholesterolemia and kidney damage in which the oxidative stress and inflammatory responses are involved in renal injury was up regulated by hypercholesterolemic condition. Oxidative stress might play a pathophysiological role due to increase of MDA levels present in higher lipid group due to impaired pro-oxidant and antioxidant mechanism. Altered physical properties of cellular membrane may facilitate the escape of free radicals from mitochondrial electron transport chain leads to lipid peroxidation and cell oxidative injury. Further hypercholesterolemia associated with oxidative modification of LDL, Protein glycation, glucose auto oxidation leads to elevated oxidative stress. The treatment with EECS and rosuvastatin significantly decrease the levels of urea, uric acid and creatinine when compare to hyperlipidemia group. Antioxidant defense enzymes SOD, CAT and GSH, protect the aerobic cells against oxygen stress and lipid peroxidation. SOD and GSH are the first line of cellular defence against oxidative injury by producing excess free radical production which is involved in the disposal of superoxide radical to hydrogen peroxide and catalase converts H_2O_2 to H_2O. Low SOD and GSH activity attribute inactivation of enzyme by ROS bringing damage to proteins. GSH is a stable enzyme inactivated by severe oxidative stress due to hyperlipidemia. The declined levels of lipid peroxidation in EECS and rosuvastatin treated rats contribute to the potential inhibitors of lipid peroxidation due to the presence of polyphenols like, flavonoids, tannins and phenolics as they are reported to exhibit antioxidant properties. The histological studies reveals that the heart sections treated/induced with cholesterol showed a marked fat cells in the heart which can increase the lipid peroxidation due to oxidative stress causes increase in the TC, TG, LDL, VLDL levels in positive control group when compare to normal group. The normal group shows decrease level of lipoproteins and show the normal architecture of the heart cells. Animals treated with Rosuvastatin showed a significant restoration of lipoproteins in the heart by maintaining ROS production whereas group IV and V animals treated with EECS preserved the architecture of heart cells by reducing the free fatty acids exhibiting antihyperlipidemic activity. Naturally occurring flavonoids and saponins can reduce the excess production of LDL and VLDL levels and increase the HDL levels in the tissues.

Table: 1 Effect of eecs on weight gain in hyperlipidemia - induced rats for 12 weeks

Parameter	Test group	Day 0	14th day	28th day
Weight gain	Normal control	122.7±3.10	125± 3.36	128.5± 3.12
	Positive control	215.5±7.00	224.8±8.21	235.8±7.22
	Standard	200.3±4.79***	171.3± 5.03***	156±4.55***
	EECS250mg/kg	204.5±5.36*	190±7.05***	174.7±5.01***
	EECS500mg/kg	193.3±3.78***	175.2±4.68***	163±4.87***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, ** P<0.01, ***P<0.001 (one way ANOVA followed by Dunnett test)

Graph I

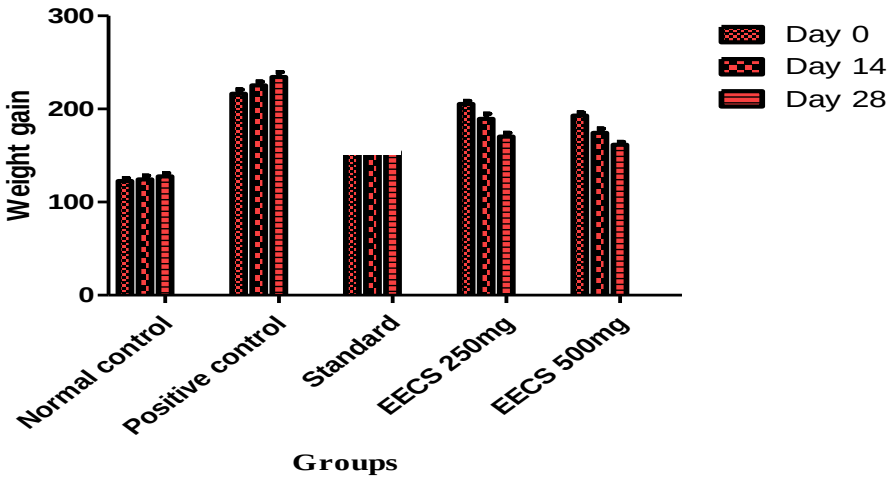
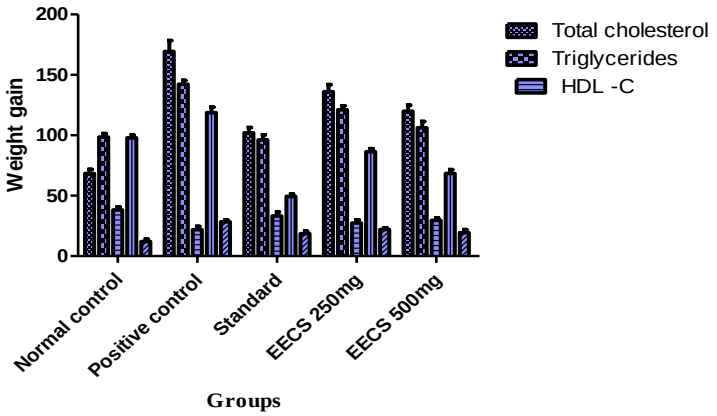


Table: 2 Effect of eecs on serum lipid profile

Groups	Serum cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal control	67.33± 3.98	57.17± 2.40	38.67± 4.8	18.00± 2.60	11.33± 2.16
Positive control	171.00±10.57	144.8± 8.18	22.67±2.80	119.3± 7.16	29.67±3.733
Standard	103.5±3.73***	94.83±3.31***	34.83±3.18***	48.90±3.55***	19.83±2.51***
EECS 250mg/kg	137.8±4.16***	120.00±4.74***	26.67± 2.60ns	88.00±3.23***	22.50±2.739**
EECS-500mg/kg	120.5±4.88***	105.8± 3.76***	30.5± 2.73*	69.33±2.58***	20.83±3.18***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, ** P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet test).

Graph II



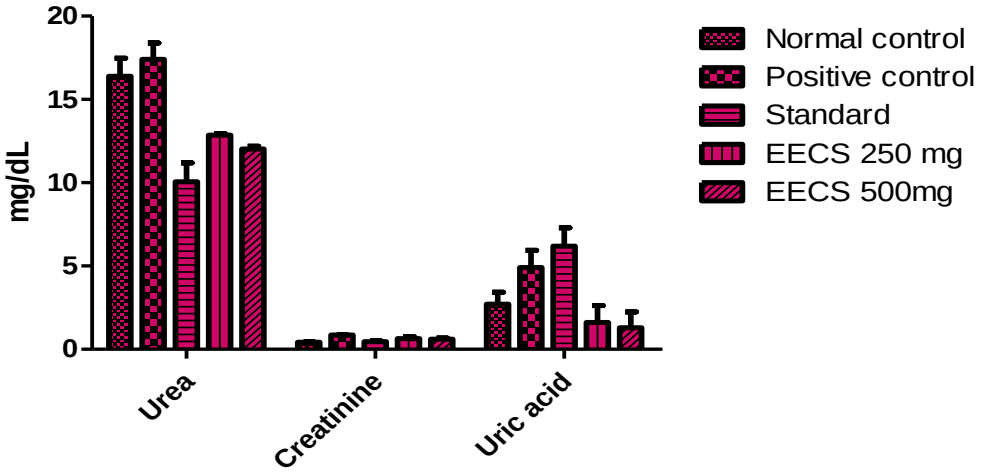
Effect of EECS on lipid profiles TC, TG, LDL, VLDL, HDL levels in cholesterol induced rats.
Values are expressed in Mean± SD for six animals.

Table: 3 Effect of eecs on serum kidney biomarkers in normal and cholesterol rats.

GROUPS	UREA (mg/dL)	CREATININE (mg/dL)	URIC ACID (mg/dL)
Normal control	16.2±2.30	0.44±0.101	2.2±0.50
Positive control	17.5±3.2	0.85±0.12	4.80±1.4
Standard	10.6±1.70***	0.45±0.108***	1.30±0.10***
EECS-250mg/kg	13.2±0.80*	0.65±0.09*	1.70±0.51***
EECS-500mg/kg	12.8±0.60*	0.62±0.07*	1.40±0.23***

Values are expressed as mean ± SD (n=6).Values were significant when compared with positive control group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet test).

Graph III



Effect of EECS on serum kidney biomarkers urea, creatinine, uric acid levels in cholesterol induced rats.

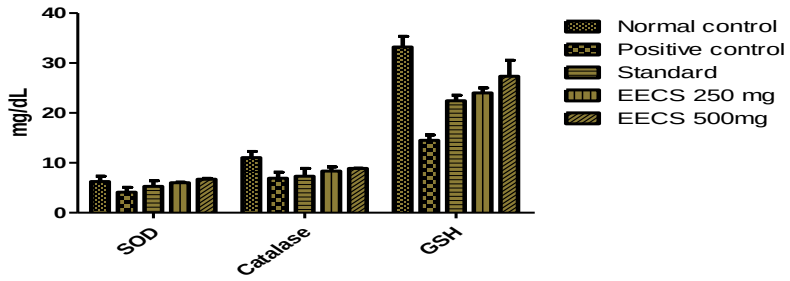
Values are expressed in Mean± SD for six animals.

Table: 3 Effect of eecs on antioxidant enzymes in control and experimental rats.

GROUPS	SOD	CATALASE	GSH
Normal control	6.90± 0.60	11.38± 0.81	34.76±1.16
Positive control	4.00± 0.61	6.31± 0.69	13.39±0.56
Standard	5.37± 0.72*	7.36± 0.64^{ns}	22.12±0.98***
EECS 250mg/kg	6.08± 0.87***	8.41± 0.75**	24.19±1.16***
EECS 500mg/kg	6.61± 0.63***	8.83± 0.60***	28.57±1.87***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet test).

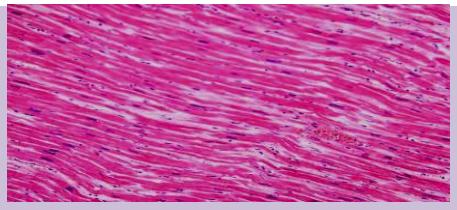
Graph IV



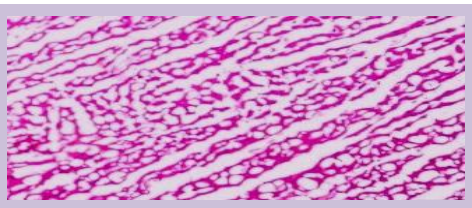
Effect of EECS on antioxidants of SOD, CAT and GSH levels in Cholesterol induced rats

Values are expressed in Mean± SD for six animals

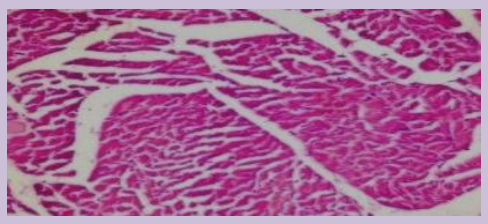
HISTOPATHOLOGICAL STUDIES



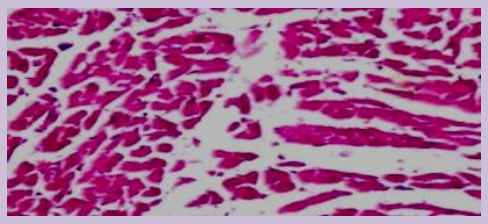
Normal control of Heart



Positive control Heart



Standard group of Heart



EECS 250mg/kg of heart

EECS 500mg/kg of heart

SUMMARY AND CONCLUSION

The findings of the study suggest that *Clerodendron serratum Linn.* is a potent drug for antihyper cholesterolemic, anti hyper tri- glycerolemic drug lowering LDL,VLDL and increasing HDL levels in all the High cholesterol diet treated groups. The mechanism has point towards inhibiting cholesterol and triglyceride synthesis. Aerial parts of *Clerodendron serratum Linn.* proved to be effective at higher doses (500mg/kg) and Rosuvastatin standard drug shows better results in decrease TC,TG, LDL and VLDL levels and increase the HDL levels compare to hyperlipidemia rats. The urea, creatinine, and uric acid levels are decreased with EECS compare to the hyperlipidemia rats. In hyper lipidemic rats, there is a decrease in antioxidant enzymes- SOD, CAT and GSH due to the free radical mechanism. The EECS group shows increase in antioxidant enzymes. Therefore, it can be recommended for further investigation to reveal the exact mechanism for hyperlipidemia activity.

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