

Hypolipidemic and antioxidant activity of ethanolic extract of *Symplocos racemosa* Roxb. in hyperlipidemic rats: An evidence of participation of oxidative stress in hyperlipidemia

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Hypolipidemic and antioxidant activity profiles of ethanolic extracts of *Symplocos racemosa* (EESR) were studied by triton-WR1339 (acute) and high fat diet induced (chronic) hyperlipidemic rat models. In both the models, a significant increase in total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and decrease in high density lipoproteins (HDL) in serum were observed. EESR (200 and 400 mg/kg) and simvastatin (10 mg/kg) administered orally reduced the elevated serum lipids (TC, TG, VLDL, LDL), restored the decreased HDL and improved the atherogenic index. In high fat diet induced hyperlipidemic model, EESR treatment prevented the increased formation of malondialdehyde (MDA) in liver, restored the depleted liver antioxidants, glutathione, superoxide dismutase, catalase significantly. The increased liver cholesterol, HMG-CoA reductase activity and body weight of hyperlipidemic rats were significantly reduced by EESR treatment. The EESR inhibited HMG-CoA reductase, a rate limiting enzyme in cholesterol biosynthesis, thereby causing hypolipidemic effects. EESR treatment also improved histoarchitecture of hepatocytes in hyperlipidemic rats. Experimental findings demonstrated anti-hyperlipidemic and antioxidant activity of EESR, which may be directly or indirectly related to its antioxidant activity. The hypolipidemic activity of EESR may be due to the presence of flavonoids phenolic compounds, phenolic glycosides and steroids.

Keywords: Hyperlipidemia, Hypolipidemia, Plant-antioxidants, *Symplocos racemosa*

Hyperlipidemia is characterized by an abnormal elevation in the major circulatory lipids and lipoproteins. The situation occurs mainly due to impaired lipid and lipoprotein metabolism which also adversely affects the pathways of cholesterol transport. The hypercholesterolemia is considered to be the most prevalent positive risk factor for initiation of atherosclerosis, which subsequently leads to cardiovascular complications^{1,2}. The lipid metabolism is largely influenced by host of risk factors including sedentary lifestyle, lack of physical activities diet enriched with cholesterol saturated fats, obesity, age and hormonal deregulation^{1,3,4}.

Increased formation of free radicals/reactive oxygen species (ROS) in general and also in hyperlipidemic conditions are known to participate in cardiac dysfunction, cardiovascular disease (CVD) progression, cardiac apoptosis and necrosis^{2,5}.

The formation of ROS in the body (intracellular) are normally controlled and modulated by endogenous defense mechanism(s). The increased formation of ROS causes depletion of antioxidants causing imbalance between antioxidants and ROS. This is a major triggering factor for the initiation of cellular damage⁶, development of atherosclerosis and CVD. Clinically, lipid lowering therapy is recommended in the prevention of primary as well as secondary cardiovascular diseases. The current anti-hyperlipidemic therapy mainly includes statins, fibrates and bile acid sequestrants that are almost inefficient to regulate lipid metabolism and also cause a number of serious adverse effects in patients.

On the other hand traditional herbal remedies and folklore medicines have evolved from ancient healing system and the core texts are enjoying a remarkable resurrection which may be due to their better compatibility with human system⁷⁻⁹. Therefore, research and development of a lipid lowering drug with antioxidant potential altogether, from natural products are the best option and will have great prospects.

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Symplocos racemosa Roxb (Family-Symplocaceae; English name: Symplocos bark and Hindi name: Lodh) is a medicinal plant widely used by traditional practitioners in the treatment of leprosy, tumors, diarrhoea dysentery, menorrhagia, inflammation and uterine disorders¹⁰. The bark of the plant contains triterpenoids namely; betulin and oleanolic acid phytosterol, 3-monoglucufuranoside of 7-O-methylleucopelargonidin^{11,12}. The phytochemical analysis indicates the presence of flavonoids, phenolic glycosides, alkaloids, triterpenoids and steroids in the ethanolic extract of *S. racemosa*¹³. Considering the nature of phytoconstituents present in stem bark of the plant, attempts have been made to investigate the hypolipidemic and antioxidant activity of ethanolic extract of *S. racemosa* (EESR) on rat models of hyperlipidemia. Further, if EESR exhibits hypolipidemic activity, efforts shall also be made to understand the mechanism(s) of hypolipidemic effect as well as the probable role of anti-oxidants in such pharmacological activity.

Materials and Methods

Drugs and Chemicals — Cholesterol, cholic acid (Loba chemie, India), Triton WR 1339 (Sigma, USA), Simvastatin (Lupin, India) were procured. Diagnostic reagents kit (Biolab, India) was used for biochemical assays. Other chemicals used were of analytical grade and obtained from Qualigens, India.

Collection, identification and extraction of plant material — The barks of *S. racemosa* were purchased from the local market of Pune. The plant material was authenticated and voucher specimen (Auth 09-131) was deposited at Agharkar Research Institute, Pune. The shade dried powdered material was macerated with ethanol (95%) for one week with intermittent shaking. The ethanolic extract of barks of *S. racemosa* (EESR) was concentrated under reduced pressure, using rotary evaporator. The dried extract was stored in a refrigerator and used for pharmacological evaluations.

Determination of phenolic and flavonoid contents in EESR — The total phenolic content (TPC), expressed as mg gallic acid equivalent (GAE)/g dry weight, was determined spectrophotometrically at 765 nm by the Folin-Ciocalteu method¹⁴. Flavonoids content, expressed as mg quercetin equivalent (QE)/g dry weight, was determined using modified colorimetric method¹⁵ and absorbance was measured at 510 nm.

Preparation of drug and extract for anti-hyperlipidemic study — EESR and simvastatin were

prepared as homogenous suspension, in 1% (w/v) carboxy methyl cellulose (CMC), freshly prior to treatment. The concentration of stock solution (drugs/mL) used was 40 mg/mL.

Antioxidant activity (invitro) — The free radicals scavenging activity of EESR was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) and the absorbance was measured at 517 nm as per Blois¹⁶.

Experimental animals and research protocol approval — Male Sprague Dawley rats (150-200 g) were procured from Intox Pvt. Ltd. Pune. The rats were housed under standard laboratory conditions, at 24 °C, and 50% RH and 12:12h L:D, cycle. Animals were fed with Amrut brand standard pellet diet (Nav Oil Mills Ltd, Chakan, Maharashtra, India) and drinking water *ad libitum*. The experimental procedures and protocol of the project was reviewed and approved (SCOP/IAEC/Approval/2009-10/05) by the Institutional Animal Ethics Committee (IAEC).

Acute oral toxicity — Swiss albino mice of either sex (25-30 g, body weight) were used for acute toxicity studies following the guidelines (AOT no. 425) suggested by Organization for Economic Co-operation and Development (OECD, 2001). The mice were observed by housing them individually in the polypropylene cages for clinical symptoms and mortality for 96 h after treatment.

Triton WR 1339 (Isooctyl-polyoxyethylene phenol) induced hyperlipidemia in rats — The administration of the non-ionic surfactant, triton WR 1339 (200 mg/kg, ip) to rats results in a biphasic elevation of plasma total cholesterol (TC) and triglyceride (TG). The biphasic nature of triton induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemic agents¹⁷.

Hyperlipidemia was induced acutely in male Sprague Dawley rats (150-200 g) (overnight fasted for 12-14 h) by administering triton WR-1339 (200 mg/kg, ip) prepared freshly in physiological saline solution. The rats were randomly divided into 5 groups (6 rats/group) and received the following treatment:

Group I - Normal control (NC) and received 1% w/v CMC (1 mL/100 g, body weight, po)

Group II - Triton control (TC) -Triton (200 mg/kg, body weight) and 30 min later received 1% w/v CMC (1 mL/100 g, body weight, po).

Group III - Triton (200 mg/kg, ip) + 30 min later received, EESR (200 mg/kg, body weight, po).

Group IV - Triton (200 mg/kg, ip) + 30 min later received, EESR (400 mg/kg, body weight, po).

Group V - Triton (200 mg/kg, ip) + 30 min later received, Simvastatin (10 mg/kg, body weight, po).

Determination of serum lipid profile — At the end of the experimental study (24 h after triton injection) the blood was collected by cardiac puncture under light ether anesthesia and the serum was separated. The serum TC¹⁸, TG¹⁹, high density lipoprotein (HDL)²⁰, atherogenic index, very low density lipoprotein (VLDL) and low density lipoprotein (LDL)²¹ were determined in serum, using readymade kits (Biolab, India); 24 h after triton injection²².

High fat diet induced hyperlipidemia in rats — Hyperlipidemia was induced in male Sprague Dawley rats (150-200 g) by maintaining them on a high fat diet (Table 1) for 45 days. The rats were randomly divided into following 5 groups (6 rats/group) and received the following treatment^{23, 24}:

Group I- Normal control (NC) maintained on normal diet throughout 45 days + 1% w/v CMC (1 mL/100 g, body weight, po) for 15 days (from 31st to 45th day of treatment period).

Group II- HFD control maintained on high fat diet throughout 45 days + 1% w/v CMC (1 mL/kg, po) for 15 days (from 31st to 45th day of treatment period).

Group III- Maintained on high fat diet throughout 45 days + EESR (200 mg/kg, po.) for 15 days (from 31st to 45th day of treatment period).

Group IV- Maintained on high fat diet throughout 45 days + EESR (400 mg/kg, po) for 15 days (from 31st to 45th day of treatment period).

Group V- Maintained on high fat diet throughout 45 days + Simvastatin (10 mg/kg, po) for 15 days (from 31st to 45th day of treatment period).

At the end of the experiment, after the last dose of treatment in groups II, III, IV and group V, rats were fasted for 12 h and the blood was collected by cardiac puncture under light ether anesthesia and then sacrificed. The liver was excised promptly, washed in cold normal saline and a part of liver tissue was preserved in 10% formalin for histopathological studies. The lipid profile in serum and antioxidant parameters in liver tissue was determined²⁵.

Measurement of body weight — During the experimental period body weight of rats were recorded at every 15 days interval.

Determination of serum lipid profile and biomarkers — TC, TG and HDL in serum were assayed using the readymade diagnostic kits (Biolab, India) by the methods described earlier¹⁸⁻²⁰. VLDL-TC and LDL-TC values were calculated using formula, (VLDL-TC = TG/5), LDL-TC = TC - (HDL-TC + VLDL-TC)²¹. Atherogenic index was calculated using formula, Atherogenic index = TC / HDL-TC²¹.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to the method described by Reitmen and Frankel²⁶. The enzyme activity was expressed as IU/L.

Determination of liver cholesterol and inhibition of HMG-CoA reductase (ex in vivo) activity — Liver homogenate 10% (w/v) was prepared in phosphate buffer (0.1M, pH 7.4) under controlled temperature (0-4 °C). Total cholesterol in liver homogenate was determined by Folch method²⁷. HMG-CoA reductase enzyme activity (ex in vivo) was assayed indirectly in liver homogenate and expressed in terms of the HMG-CoA and mevalonate ratio²⁸.

Evaluation of antioxidant activity — Liver was washed and homogenized in chilled 0.15 M KCl ice bath, using homogenizer, to obtain 10% (w/v) liver homogenates. The homogenates were centrifuged at 800 g for 10 min at 4 °C and used for the determination of lipid peroxide, GSH, CAT, and SOD. The quantitative assay of lipid peroxide was performed by determining the concentration of thiobarbituric acid reactive substances (TBARS) in liver using the method of Ohkawa and Yogi²⁹. The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as nmol/mg of protein. Glutathione was estimated in the

Table1— Composition (%) of high fat diet used for inducing hyperlipidemia in rats

Sr. no.	Content	Per 100 g of food pellet
1	Sodium chloride	0.5
2	Cholic acid	0.5
3	Cholesterol	1
4	Egg yolk	8
5	Gram flour	20
6	Coconut oil	20
7	Corn flour	20
8	Wheat flour	30

liver homogenates using DTNB by the method of Buetler *et al.*³⁰. The absorbance was read at 412 nm and results were expressed in terms of μmol of GSH/mg of protein. Catalase activity was assayed by spectrophotometric method³¹, for determining the breakdown of H_2O_2 at 240 nm in specific time interval and expressed in terms of $\mu\text{mol}/\text{min}/\text{mg}$ of protein. SOD activity was assayed³²; the activity of SOD was inversely proportional to the concentration of its oxidation product adrenochrome, which was measured spectrophotometrically at 320 nm and expressed in terms of U/mg of protein.

Histopathological studies — The liver samples were fixed in 10% (v/v) formalin and were embedded in paraffin wax and cut into 4 - 6 μm thick section on microtome. The sections were stained using haematoxylin and eosin, and were observed for histoarchitecture alterations like perivenular inflammatory changes, mononuclear cell infiltration within lobules and portal tracts and necrotic damage to hepatocytes structures.

Statistical analysis — Results were expressed as the mean \pm SE. The results of different treatments were subjected to statistical analysis using one way ANOVA followed by Dunnet's multiple comparison tests. The $P < 0.05$ was considered statistically significant.

Results

Total phenolic and flavonoids content in EESR — The total phenolic and flavonoids content were found to be 57.07 ± 6.04 (mg GAE/g, dry basis) and 128.3 ± 7.10 (mg QE/g, dry basis) of six independent assays, respectively.

Antioxidant activity (in vitro) — The DPPH free radical scavenging effects of the EESR was expressed

as IC_{50} values. EESR was capable of scavenging DPPH free radicals. The scavenging activity of the EESR increased with its concentration. The IC_{50} value of the EESR was found to be $120.86 \mu\text{g}/\text{mL}$.

Acute oral toxicity — Administration of EESR at different doses up to 4000 mg/kg, body weight, po did not elicit toxic clinical symptoms or gross behavioural changes, either immediately or during 96 h observation period. None of the doses produced mortality till the end of study period. Hence the LD_{50} was considered to be more than 4000 mg/kg. Therefore, doses of 200 and 400 mg/kg were selected for anti-hyperlipidemic studies, which were 1/10th and 1/20th of the highest dose studied.

Triton WR 1339 induced hyperlipidemia in rats — The administration of triton WR 1339 to rats induced a significant elevation of serum TG, TC, VLDL-TC, LDL-TC, atherogenic index at 24 h and decrease in HDL-TC. EESR treatment decreased the elevation of serum TG, TC, VLDL-TC, LDL-TC and atherogenic index significantly, whereas serum HDL-TC was restored insignificantly (Table 2).

High fat diet induced hyperlipidemia in rats — The rats maintained on HFD for 45 days showed significant increase in body weight as compared to rats on normal diet. Hyperlipidemic rats treated with EESR and simvastatin reduced body weight significantly (Table 3). The HFD rats treated with EESR and simvastatin for 15 days showed a significant decrease in serum TC, TG, LDL-TC, VLDL-TC, atherogenic index, and increased serum HDL-TC levels (Table 4).

The high hepatic cholesterol levels were observed in HFD rats may be due to its accumulation.

Table 2— Effect of EESR on Triton WR 1339 induced hyperlipidemia in rats
[Values are mean \pm SEM of 6 animals. Figures in parentheses are % increase (+) or decrease (-)]

Treatment and dose (mg/kg, po)	TC (mg/dL)	TG (mg/dL)	HDL-TC (mg/dL)	VLDL -TC (mg/dL)	LDL-TC (mg/dL)	Atherogenic index
Normal control (1% w/v CMC)	82.63 \pm 3.56	86.06 \pm 3.23	40.36 \pm 0.62	17.21 \pm 0.64	25.06 \pm 0.95	2.04 \pm 0.11
Triton control (1% w/v CMC)	311 \pm 5.37 [@] (+276)	411.8 \pm 7.49 [@] (+378)	37.71 \pm 0.68 [¥] (-7)	82.36 \pm 1.5 [@] (+ 378)	190.93 \pm 2.15 [@] (+662)	8.25 \pm 0.13 [@] (+ 304)
EESR (200)	202.5 \pm 8.37 ^{**} (- 35)	311 \pm 9.27 ^{**} (- 24)	39.96 \pm 0.51 (+ 6)	62.27 \pm 1.85 ^{**} (- 24)	100.27 \pm 3.17 [#] (- 47)	5.31 \pm 0.26 ^{**} (- 36)
EESR (400)	184.3 \pm 5.74 ^{**} (-41)	268 \pm 8.96 [#] (- 35)	42.2 \pm 0.72 (+ 12)	53.46 \pm 1.72 [#] (- 35)	88.64 \pm 2.55 [#] (- 53)	4.36 \pm 0.83 [#] (- 47)
Simvastatin (10)	99.41 \pm 3.40 [#] (- 68)	213 \pm 10.05 ^{**} (- 48)	46.16 \pm 0.69 [#] (+ 22)	42.59 \pm 2.01 [#] (- 48)	10.66 \pm 0.35 [#] (- 94)	2.15 \pm 0.07 [#] (- 74)

P Values: [@] $P < 0.001$ compared to NC group, [#] $P < 0.001$, ^{**} $P < 0.01$ compared to triton control group [¥] $P < 0.05$ compared to NC group.

Table 3— Effect of EESR on body weight in high fat diet induced hyperlipidemic rats
[Values are mean \pm SEM of 6 animals. Figures in parantheses are % increase (=) or decrease (-)]

Treatment and dose (mg/kg, po)	Body weight (g)			
	Day 0	Day 15 th	Day 30 th	Day 45 th
Normal control (1% w/v CMC)	223 \pm 1.85	226 \pm 1.98	230 \pm 1.99	235 \pm 1.94
HFD control (1% w/v CMC)	226 \pm 1.87	244 \pm 1.59 [@]	265 \pm 2.04 [@]	295 \pm 5.03 [@] (+26)
EESR (200)	227 \pm 2.50	247 \pm 2.09	274 \pm 4.30	273 \pm 4.79 [*] (-7)
EESR (400)	219 \pm 1.63	249 \pm 3.67	281 \pm 3.88	266 \pm 2.91 ^{**} (-10)
Simvastatin (10)	225 \pm 1.68	255 \pm 1.69	286 \pm 2.15	271 \pm 2.90 [*] (-8)

P Values: [@] < 0.001 compared to NC group. ^{*} <0.05, ^{**} <0.01 compared to HFD control group.

Table 4— Effect of EESR on lipid profile in high fat diet induced hyperlipidemic rats
[Values are mean \pm SEM of 6 animals. Figures in parantheses are % increase (+) or decrease (-)]

Treatment and dose (mg/kg,p.o.)	TC (mg/dL)	TG (mg/dL)	HDL-TC (mg/dL)	LDL-TC (mg/dL)	VLDL-TC (mg/dL)	Atherogenic index
Normal control (1% w/v CMC)	96.97 \pm 1.98	80.27 \pm 1.88	41.35 \pm 0.71	39.45 \pm 2.52	16.17 \pm 0.27	2.32 \pm 0.073
HFD control (1% w/v CMC)	300 \pm 3.01 [@] (+ 209)	224.9 \pm 2.51 [@] (+ 180)	30.96 \pm 0.73 [@] (- 25)	224.2 \pm 3.78 [@] (+ 468)	44.38 \pm 0.72 [@] (+ 174)	9.72 \pm 0.28 [@] (+ 319)
EESR (200)	173.8 \pm 3.61 [#] (- 42)	119.2 \pm 2.59 [#] (- 47)	36.68 \pm 0.70 [#] (+ 18)	113.3 \pm 4.48 [#] (- 49)	23.84 \pm 0.51 [#] (- 46)	4.74 \pm 0.17 [#] (- 51)
EESR (400)	143.8 \pm 8.30 [#] (- 52)	114 \pm 1.05 [#] (- 49)	40.15 \pm 1.06 [#] (+ 30)	80.89 \pm 9.46 [#] (-64)	22.8 \pm 0.21 [#] (- 49)	3.61 \pm 0.29 [#] (-63)
Simvastatin (10)	109.6 \pm 3.16 [#] (- 63)	86.24 \pm 1.37 [#] (- 62)	45.45 \pm 0.89 [#] (+ 47)	46.87 \pm 3.64 [#] (- 79)	17.25 \pm 0.25 [#] (-61)	2.51 \pm 0.09 [#] (- 74)

P values: [@] < 0.001 compared to NC group, [#] < 0.001 compared to HFD control group.

The treatment with EESR and simvastatin, for 15 days, significantly reduced hepatic cholesterol (Table 6). Since the ratio of HMG-CoA and mevalonate is inversely proportional to HMG-CoA reductase activity, an increased ratio indicates the decreased enzyme activity. EESR and simvastatin treatment showed inhibition of HMG-CoA reductase activity dose dependently (Table 5).

Antioxidant activity — The liver malondialdehyde (MDA) in HFD rats was elevated significantly. EESR and simvastatin treatment prevented significantly the increased liver MDA of HFD rats. Further, EESR and simvastatin treatment also restored the depleted liver GSH, SOD and CAT significantly in HFD rats (Table 6).

Histopathological studies — In HFD induced hyperlipidemic model, a significant fatty infiltration, granular degeneration and increased mononuclear cell

infiltration within lobules was observed in the liver sections of HFD control rats as compared to normal rats (Fig. 1). A dose dependant and marked decrease in the fatty infiltration and mononuclear cell infiltration within lobules of liver sections of HFD rats were observed in EESR (200 and 400 mg/kg) and Simvastatin (10 mg/kg) treated rats respectively. A marked increase in mononuclear cell infiltration within portal tracts of HFD control rats was also observed, which was found to be reduced with the treatment of EESR (400 mg/kg) and Simvastatin (10 mg/kg). HFD control rats showed moderate hepatocytes swelling and necrosis, and 15 days treatment of EESR (200 mg/kg) decreased the hepatocytes swelling as well as necrosis. EESR (400 mg/kg) and Simvastatin (10 mg/kg) treatment to HFD rats prevented hepatocytes swelling and necrosis.

Discussion

In the present study, triton induced hyperlipidemic model was used as an acute model for preliminary and rapid screening of hypolipidemic activity of EESR. Moss and Dajani³³ reported that, triton (a surfactant) blocks the clearance of triglyceride rich lipoproteins from circulation, by extra-hepatic tissues, causing an increased circulating lipid. Triton WR-1339 has been widely used to induce hyperlipidemia in experimental animals³⁴ and routinely used for screening of hypolipidemic agents. The hypolipidemic activity of EESR may be due to the increased removal of triglycerides and lipoproteins from blood circulation, leading to decrement in circulating serum lipids. Alternatively, EESR may also affect metabolic and excretory phases of lipids causing depletion of increased lipids in the serum.

The biological activities of flavonoids have been attributed largely due to their powerful antioxidant

properties. Flavonoids are known to act in several ways: (a) direct quenching of ROS, (b) chelating of metal ions and (c) activation of membrane bound antioxidants³⁵. Flavonoids are known to be involved in modulation of lipid peroxidation, atherogenesis and thrombosis³⁶. Many of the biological activities of flavonoids are also linked to their hypolipidemic properties³⁷. Flavonoids and polyphenolic compound are potent free radical scavengers and are reported to modulate the activities of various defensive enzyme systems, largely due to their interactions with various endogenous biomolecules³⁸. Phenolic phytochemicals due to their phenolic ring and hydroxyl substituent, exhibit strong antioxidant activity (e.g. neutralization of hydroxyl and other free radicals) like vitamin E. It is, therefore, believed that dietary phenolic antioxidants can scavenge harmful free radicals, and thus, inhibit their oxidative reactions with vital biological molecules³⁹. *Symplocos racemosa* contain

Table 5 — Effect of EESR on Liver cholesterol, and HMG CoA reductase activity in high fat diet induced hyperlipidemic rats
[Values are mean \pm SEM of 6 animals. Figures in parantheses are % increase (+) or decrease (-)]

Treatment and dose (mg/kg, po)	Liver cholesterol (mg/g)	HMG-CoA / Mevalonate-ratio
Normal control (1% w/v CMC)	12.56 \pm 0.94	2.83 \pm 0.012
HFD control (1% w/v CMC)	41.37 \pm 0.84 [@] (+ 229)	1.13 \pm 0.079 [¥] (- 60)
EESR (200)	29.15 \pm 0.70 [#] (- 30)	1.78 \pm 0.018 [¥] (+ 57)
EESR (400)	22.47 \pm 0.89 [#] (- 46)	1.98 \pm 0.05 [#] (+ 75)
Simvastatin (10)	17.82 \pm 0.93 [#] (- 57)	2.91 \pm 0.028 [#] (+ 157)

P values: ¥ < 0.01 compared to NC group, @ < 0.001 compared to NC group, # < 0.001 compared to HFD control group.

Table 6— Effect of EESR on liver TBARS, GSH, CAT, SOD level in high fat diet induced hyperlipidemic rats
[Values are mean \pm SEM of 6 animals. Figures in parantheses are % increase (+) or decrease (-)]

Treatment and dose (mg/kg, po)	TBARS (nmolMDA/mg protein)	GSH (ng/mg protein)	CAT (μ g/mg protein)	SOD (U/mg protein)
Normal control (1% w/v CMC)	2.37 \pm 0.26	20.16 \pm 0.64	235.2 \pm 1.76	41.33 \pm 0.85
HFD control (1% w/v CMC)	5.62 \pm 0.23 [@] (+ 137)	10.53 \pm 0.50 [@] (-48)	115.6 \pm 1.95 [@] (- 51)	21.09 \pm 0.44 [@] (- 49)
EESR (200)	4.32 \pm 0.18 ^{**} (- 23)	13.91 \pm 0.39 [#] (+ 32)	160.8 \pm 1.83 [#] (+ 39)	30.51 \pm 0.52 ^{**} (+ 45)
EESR (400)	3.55 \pm 0.26 [#] (- 37)	15.21 \pm 0.34 [#] (+ 44)	188.6 \pm 1.51 [#] (+ 63)	32.18 \pm 0.65 [#] (+ 53)
Simvastatin (10)	2.71 \pm 0.24 [#] (- 52)	17.6 \pm 0.48 [#] (+ 67)	219.1 \pm 2.47 [#] (+90)	37.3 \pm 0.62 [#] (+ 77)

P values: @ < 0.001 compared to NC group, # < 0.001 compared to HFD control group, ** < 0.01 compared to HFD control group.

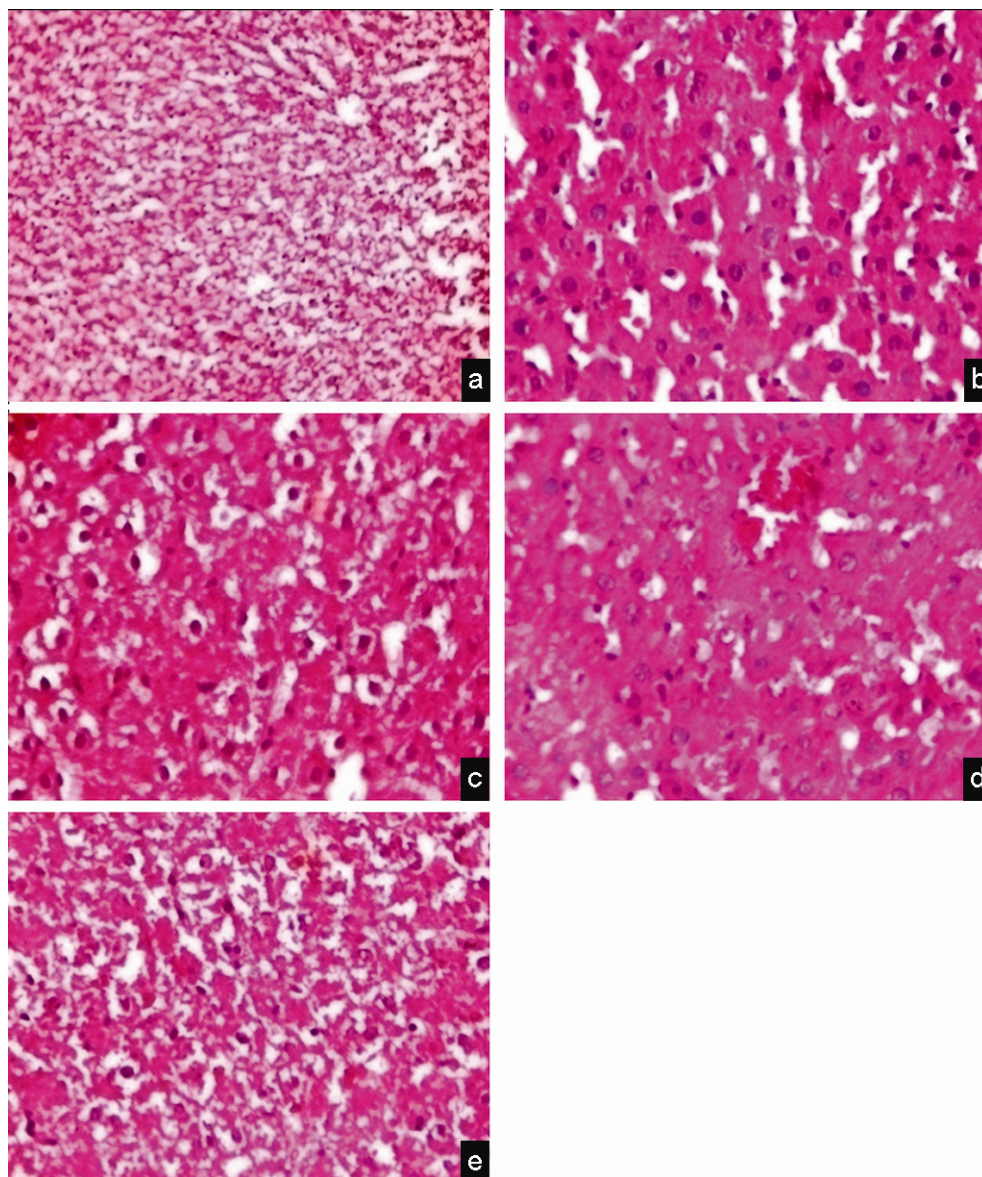


Fig. 1—Transverse section of hepatic tissue of rats (a) Normal control group showing normal architecture; (b) high fat diet (HFD) control group; (c) HFD+EESR (200 mg/kg); (d) HFD+EESR (400 mg/kg); (e) HFD + Simvastatin (10 mg/kg). H&E 100 \times]

flavonoids, total phenolic compounds, phenolic glycosides and steroids, which may act either individually or synergistically to elicit antioxidant activity, which may ultimately manifest in the form of anti-hyperlipidemic effect.

The chronic hyperlipidemia leads to atherosclerosis which is implicated in the pathophysiology of coronary artery disease and myocardial ischemia. High fat diet induced hyperlipidemia in rats is an established and documented animal model to evaluate effects of anti-hyperlipidemic drugs. The chronic hyperlipidemia finally alters morphological and hemodynamic functions of heart as well as liver⁴⁰. A

significant increase in body weight was observed in HFD fed rats. Treatment with EESR and standard simvastatin reduced increased body weight of hyperlipidemic rats.

In the present study, the EESR showed a significant hypolipidemic activity in HFD induced hyperlipidemic rat model, the activity is comparable to that of standard drug, Simvastatin, used clinically in the management of cardiovascular diseases. HFD caused a significant rise of serum TC, TG, LDL-TC, VLDL-TC and concomitant decrease in serum HDL-TC. Treatment of HFD fed rats with EESR (200 and 400 mg/kg) and Simvastatin (10 mg/kg), significantly

decreased the elevated TC, TG, LDL-TC, VLDL-TC and restored the decreased HDL-TC in serum. Treatment with EESR also reduced significantly the atherogenic index, which is an important and sensitive indicator to determine the cardiac risk as claimed by NCEP guidelines⁴¹.

The liver plays a central role in regulating the cholesterol and plasma LDL metabolism through the secretion of cholesterol and bile acids. Consumption of high fat diet increases cholesterol input in liver and hepatic synthesis of VLDL and LDL-cholesterol^{42,43}, resulting in increased accumulation of cholesterol in liver⁴⁴. Treatment with EESR reduced liver cholesterol content significantly.

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, i.e. increased ratio indicates decreased enzyme activity and vice-a-versa²⁸. Simvastatin has been reported to inhibit HMG-CoA reductase and thereby lowers lipid level⁴⁵. In the present study, EESR treatment inhibited HMG-CoA reductase dose dependently. Such inhibitory activity by EESR on HMG-CoA reductase may be responsible for the reduction of the liver cholesterol observed in the present study. Hence it is presumed that the mechanism of action of anti-hyperlipidemic activity of EESR resembles to that of simvastatin.

It is documented that intake of high fat diet causes abnormal rise in lipid and lipoprotein levels and also increases the lipid peroxidation process that may lead to oxidative damage due to the formation of generation of excessive free radicals in the liver⁴⁶. Formation of highly reactive free radicals during hyperlipidemia causes oxidative stress which accelerates the development and progression of atherogenesis. The decreased lipid peroxidation may be one such avenue that can reduce the chances of atherosclerosis development due to hyperlipidemia⁴⁷. The increased formation of malondialdehyde (MDA) in rats fed with HFD clearly point out that, hyperlipidemia is responsible for the enhanced lipid peroxidation process. In the present experiments, it was observed that oral administration of EESR and Simvastatin to hyperlipidemic rats significantly inhibited MDA formation in liver. The reduced GSH is one of the most abundant non-enzymatic antioxidant present in liver and other tissues. The decreased GSH in the HFD rats may be due to

increased utilization of GSH to augment the activities of antioxidant enzymes, GPx, SOD and CAT⁴⁸. The restoration of depleted liver GSH was observed both in EESR and Simvastatin treated rats. It is possible that an increased biosynthesis of GSH or improved glutathione reductase activity may have been accomplished in presence of EESR. Hyperlipidemia may have impaired antioxidant defense system, and that must have lead to the depletion of SOD and CAT activity⁴⁹. Treatment of EESR and simvastatin enable to modulate, neutralize or scavenge ROS/oxidative free radicals and thus, ultimately helps in amelioration of oxidative stress, which in turn restored the depleted antioxidant enzymes, SOD and CAT. Occurrence of oxidative stress on cellular system because of genesis of hyperlipidemia perhaps has been reversed by the phytochemical(s) present in EESR, that may have resulted in hypolipidemic effect. However, the other probable mechanism(s) such as effect on cholesterol synthesis, metabolism and its excretion, by the phytochemical(s), especially sterols and others; cannot be ignored.

In conclusion, the present study demonstrate that the administration of EESR elicited promising anti-hyperlipidemic activity, that may be attributed to the phytochemical(s) present, which has reversed the genesis of hyperlipidemia, manifested due to oxidative stress on cellular system. This needs further isolation of the active phytoconstituents and to investigate their anti-hyperlipidemic activity in order to confirm the precise role of antioxidants in triggering hypolipidemic activity.

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