

RESEARCH ARTICLE

EVALUATION OF ANTIHYPERLIPIDAEMIC EFFECT OF CEDRELA TOONA ROXB. FRUITS

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ABSTRACT

Fruit extracts of *Cedrela toona* Roxb. were evaluated for their antihyperlipidaemic effect in swiss albino female rats. High Cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2% or 30 %, with standard powdered standard animal food. The diet was placed in the cage carefully and was administered for seven days. Methanol, Chloroform, and Aqueous extracts of *Cedrela toona* fruits were administered orally at a dose of 250 mg/kg body wt to Hyperlipidaemic rats. After seven days, blood samples were collected from the tail vein after 8 hr fast and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically for Cholesterol, triglyceride and HDL-C was estimated using diagnostic kits which were procured from Lab-Care Diagnostics (India) Pvt. Ltd.- Mumbai (India). Results showed that methanolic extract and aqueous extract had significant effect in hyperlipidemic rats.

Keywords: *Cedrela toona*, Hyperlipidaemia, Fruit, Cholesterol, Triglyceride.

INTRODUCTION

Literature survey reveals that *Cedrela toona* Roxb. is medium sized to large deciduous tree with brown to grey scaly bark. Leaves 15 – 45 cm long usually paripinnate but sometimes with a terminal leaflet in juvenile growth, leaflets mostly 8-20, ± ovate, often falcate, 4-15 cm long, 15-50 mm wide, apex acuminate, base strongly asymmetric, margins entire, mostly glabrous, domatia present as small hair – tufts; petiole 4-11 cm long, petiolules 5-12 mm long. Panicles 20-40 cm long. Petals 5-6 mm long, white. Capsule ellipsoid, 10-20 mm long, 6-8 mm diameter; seeds winged at both ends^{1,2,3,4}. Traditionally the bark is astringent, antidiarrhoeic, antiperiodic⁵. Flowers are emmenagogue, leaf is spasmolytic, hypoglycaemic and antiprotozoal⁶. Bark and heartwood yielded tetraterpenoids, including toonacillin. Heartwood also gave a coumarin geranyl geraniol as its fatty esters. Toonacillin and its 6 – hydroxyl derivatives are antifungal⁵. It is useful in chronic dysentery, ulcer, leprosy, cures fever, headache, blood complaints (Ayurveda), cardiogenic, aphrodisiac, anthelmintic; good for scabies and expectorant (Yunani)^{6,7}.

Phytochemical studies reported the presence of Cedrelone, isolated from the benzene extract of the heartwood of the *Cedrela toona* Roxb^{9,10}, sesquiterpene, cycloartene stigmaterol, campesterol, apotirucallene, tirucallene, catechin, proanthocyanidin, leucoanthocyanidin, toonacillin, 6-acetoxy toonacillin, toonacilid, geranyl geraniol, δ-cadinene, calamenene, α-calacorene, siderin, deoxycedrelone¹⁸. Cedrelone, isolated from the benzene extract of heartwood of *Toona ciliata*, on photooxidation yield; 3[14β,15β,22β,23β-diepoxy-6-hydroxy-6-hydroxy-1,5,20(22)-meliatriene-2,7,21-trione], along with product 4[14β,15β-epoxy-6,23-dihydroxy-1,5,20(22)-meliatriene-2,7,21-trione]¹¹. 12α-hydroxystigmat-4-en-3-one: a new bioactive steroid isolated from the petroleum ether extract

of *Toona ciliata* (Meliaceae) along with the two known steroid and three C-methyl coumarins¹². 5-methylcoumarins isolated from the dried and powdered stem bark of *Toona ciliata*, extracted successively with light petroleum ether (40-60°), dichloromethane and methanol in soxhlet apparatus¹³. Limonoids i.e. Toonaciliatins were reported from leaves and stem of *Toona ciliata*¹⁴. Siderin, a natural coumarin was isolated from the methanolic extract of the leaves of *Toona ciliata* with the help of column chromatography¹⁵. Toonafolin, a tetranortriterpenoid Blactone isolated from the ether extract of leaves of *Toona ciliata*. Polyynes isolated from the ethylacetate extract of the leaves of *Toona ciliata*¹⁶. Seven new compounds were isolated from the petrol and chloroform extract of the trees of *Toona ciliata*, and their structure were identified as 3-Acetoxy 17-furan-3-yl-1-hydroxy-1,4,4,10,13-penta-methyl-12-oxo-tetradecahydro-16,20-dioxacyclopropa[14,15]cyclopenta[α]phenanthrene-7-carboxylic acid methyl ester, beta sitosterol, stigmaterol, n-C35H72, palmitic acid, n-C20H42, 3-(3-Propyl-1,1,3,1-tercyclohexan-3-yl)-propan-1-ol¹⁷. 9,10-dihydrophenanthrenes isolated from the dichloromethane extract of the root of *Toona ciliata*¹⁸. One new limonoid, toonaciliatone A, and one new tirucallane type triterpenoid, toonaciliatine A, along with three known compounds, methyl-3β-acetoxy-1-oxomelic-14(15)-enate, perforin A, and cholest-14-ene-3,7,24,25-tetrol-21,23-epoxy-21-methoxy-4,4,8-trimethyl-3-(3-methyl-2-butenate), were isolated from the leaves of *Toona ciliata*^{19,20}.

Plant also possess antioxidant^{21,22}, Antiulcer^{23,24}, Analgesic²⁵, Antifungal²⁶, Antimicrobial^{27,28}, Anti feedant, Anti tumor²⁹ activity and cytotoxicity²⁹. The present study is designed to explore the anti diabetic effect of various

extracts of leaves of the plant *Cedrela toona* Roxb. belonging to Family Meliaceae. The present study is designed to explore the anti hyperlipidaemic effect of various extracts of fruits of the plant *Cedrela toona* Roxb. belonging to Family Meliaceae.

MATERIAL AND METHODS

Chemicals

All the chemicals used were of analytical grade and purchased from the Chemco, Rajkot, Gujarat, India and Sd Fine Chem. Limited Mumbai, India.

Plant collection and identification

The fruits of the plant were collected from the Paritosh Herbals, Dehradun in the month of October 2011. The plant was identified and authenticated as *Cedrela toona* Roxb. (Family: Meliaceae) by Dr. M. S. Jangid, Department of Botany at Sir P. T. Science College, Modasa, Gujarat, India where a voucher specimen has been deposited.

Processing of collected plant sample

The collected plant material was air-dried for two weeks and then powdered using mortar and pestle. The powder obtained was stored in air tight for use in phytochemical analysis and determination of pharmacopoeia standards³¹.

Animals³¹

Swiss albino/Sprague Dawely female rats weighing 150-200-250 gm were acclimatized to the experimental room having temperature $23 \pm 2^\circ\text{C}$, controlled humidity conditions, and 12:12 hour light and dark cycle. Animals were caged in polypropylene cages in a group with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee, B. Pharmacy College, Rampura – Kakanpur, Gujarat, India (IAEC/RAMPH/04/2011-12).

Induction of hyperlipidemia^{32,33}

High Cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2% or 30 %, with standard powdered standard animal food. The diet was placed in the cage carefully and was administered for seven days.

Instruments

The following instruments were used in the study.

- UV spectrophotometer (Shimadzu 1650 PC)
- Centrifuge (Remi)
- Sonicator (Enertech Lab)

Preparation of the Extracts³⁴

100g of each of air-dried powdered material of leaves, stems and fruits of *Cedrela toona* Roxb. was successively

extracted with the following solvents of increasing polarity in a soxhlet apparatus.

- petroleum ether ($60^\circ - 80^\circ\text{C}$)
- hexane
- Acetone
- methanol
- distilled water

All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 50°C . Each time before extracting with the next solvent, the marc was dried in an air oven below at 50°C . The marc was finally macerated with water for 24 hours to obtain the aqueous extract. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The liquid extracts obtained with different solvents were collected. The extracts were dissolved in water by preparing dose of 1 gm/kg.

Treatment protocol

The experimental animals were divided into six groups, six animals in each group

Group-1: Normal

Group-2: High cholesterol diet control

Group-3: High cholesterol diet treated with Petroleum ether extract of *Cedrela toona* Roxb. [1gm/Kg body weight, p.o.]

Group-4: High cholesterol diet treated with Acetone extract of *Cedrela toona* Roxb. [1gm/Kg body weight, p.o.]

Group-5: High cholesterol diet treated with Methanol extract of *Cedrela toona* Roxb. [1gm/Kg body weight, p.o.]

Group-6: High cholesterol diet treated with Water extract of *Cedrela toona* Roxb. [1gm/Kg body weight, p.o.]

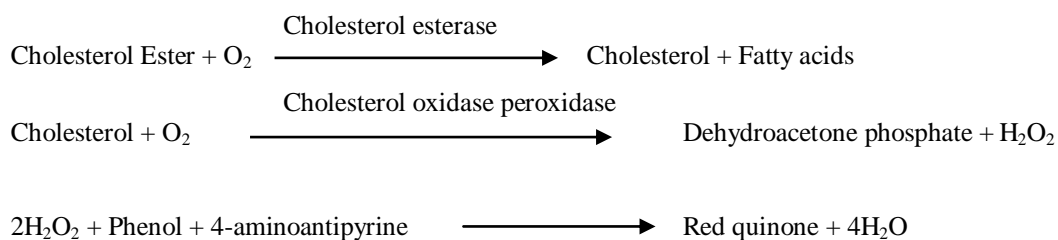
Treatment was given daily for seven days orally.

Blood sample collection and analysis^{32, 33}

After seven days, blood samples were collected from the tail vein after 8 hr fast and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically for Cholesterol, triglyceride and HDL-C was estimated using diagnostic kits which were procured from Lab-Care Diagnostics (India) Pvt. Ltd.- Mumbai (India).

Cholesterol

Principle



The intensity of the red complex (red quinone) formed during the reaction is directly proportional to the cholesterol concentration in the sample and is measured at 500nm.

Procedure

Reagents were reconstituted as described in the leaflet supplied along with the kit. 10 µl of serum samples, distilled water serving as control and standard triglyceride (200 mg/dl) serving as standard were mixed well with 1.0 ml reconstituted reagent i.e. enzyme/chromogen mixture. They were incubated at 37°C for min and absorbance was read against blank at 500nm.

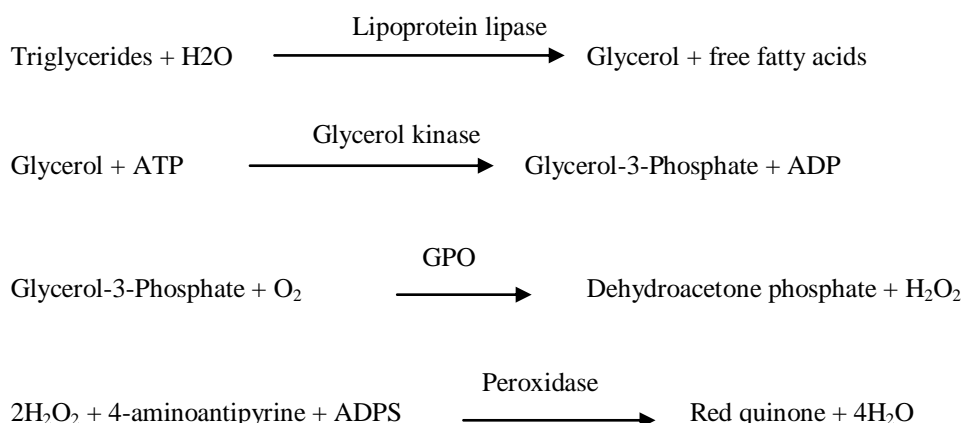
Calculation

$$\text{Serum cholesterol (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 200$$

Triglyceride

Principle

Triglycerides are enzymatically hydrolyzed to glycerol according to the following reactions



GPO = Glycerol-3-Phosphate Oxidase

ADPS= N-Ethyl-N-Sulfopropyl-n-anisidine

The intensity of the red complex (red quinone) complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and is measured at 546nm. The final colour is stable for at least 30 min.

Procedure

Reagents were reconstituted as described in the leaflet supplied along with the kit. 10 µl of serum samples, distilled water serving as control and standard triglyceride (200 mg/dl) serving as standard were mixed well with 1.0 ml reconstituted reagent 1 i.e. enzyme/chromogen mixture. They were incubated at 37°C for min and absorbance was read against blank at 546nm.

Calculation

$$\text{Serum triglyceride (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 200$$

Principle

Chylomicrons, VLDL, and LDL fractions in serum or plasma are separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen 4-amino antipyrine/phenol.

Procedure

Reagents were reconstituted as described in the leaflet supplied along with the kit. 0.2 ml of serum sample was mixed well with 0.2 ml of precipitating reagent (Reagent 2) and centrifuged at 3500-4000 for 10 min. Supernatant 20 µl and 1 ml of reconstituted reagent 1 was added. In case on blank 1 ml reconstituted reagent 1 was taken. Absorbance of test samples was measured against reagent blank at 500nm.

Calculation

$$\text{Serum HDL-C (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 50 \times 2$$

VLDL, LDL, HDL-ratio and Atherogenic index were calculated by using the formula as mentioned below:

$$\text{VLDL-C} = \frac{\text{Total serum triglycerides}}{5}$$

$$\text{LDL-C (mg/dl)} = \text{Total serum cholesterol} - \frac{\text{Total serum triglycerides}}{5} - \text{HDL-C}$$

$$\text{HDL ratio} = \frac{\text{HDL-cholesterol} \times 100}{\text{Total serum cholesterol} - \text{HDL-C}}$$

$$\text{AI} = \frac{\text{Total serum triglycerides}}{\text{Total serum HDL-C}}$$

Statistical Analysis

Results are presented as mean \pm SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey test. Data were considered statistically significant at P value \leq 0.05.

RESULT AND DISCUSSION

Effect of one week treatment with different extract at a dose 250 mg/kg in high cholesterol diet induced hyperlipidaemia in rats. PE: Petroleum Ether Extract, CE : Chloroform Extract, ME : Methanolic Extract, AE : Aqueous Extract

Table 1: Effect of various extracts on LDL, VLDL, HDL – Ratio and Atherogenic Index

Sr. no.	Group	LDL-C	VLDL	HDL-Ratio	Atherogenic Index
1	Normal	6.21 \pm 6.57	17.49 \pm 0.49	215.72 \pm 70.51	2.08 \pm 0.10
2	Control	447.39 \pm 21.66	36.35 \pm 1.29	4.42 \pm 0.35	8.57 \pm 0.09
3	PE	373.70 \pm 25.86	25.33 \pm 0.54	7.15 \pm 0.47	4.48 \pm 0.18
4	CE	256.83 \pm 5.53	19.74 \pm 2.73	12.50 \pm 2.43	2.85 \pm 0.67
5	ME	266.50 \pm 4.98	14.67 \pm 0.88	12.91 \pm 2.43	2.02 \pm 0.43
6	AE	234.62 \pm 0.15	17.74 \pm 0.49	15.58 \pm 2.16	2.26 \pm 0.54

Table 2: Effect of various extracts on Serum cholesterol, Triglyceride and HDL - C

Sr. no.	Group	Serum Cholesterol	Triglyceride	HDL-C
1	Normal	65.82 \pm 1.90	87.44 \pm 2.45	42.12 \pm 1.20
2	Control	378.73 \pm 5.00	181.80 \pm 6.47	21.23 \pm 0.91
3	PE	320.51 \pm 6.58	126.67 \pm 2.71	33.31 \pm 1.96
4	CE	311.13 \pm 10.28	98.71 \pm 13.65	34.56 \pm 2.01
5	ME	317.47 \pm 7.85	73.33 \pm 4.43	36.29 \pm 1.98
6	AE	291.64 \pm 4.56	88.72 \pm 2.45	39.28 \pm 4.21

Serum cholesterol (SC)

High cholesterol diet rats exhibited higher cholesterol levels as compared to normal rats (Fig 1). Treatment with ME and AE significantly decreased elevated cholesterol levels in hyperlipidemic rats.

Serum triglyceride

High cholesterol diet rats exhibited significantly higher triglyceride (Fig 2) levels as compared to normal control rats. Treatment with ME and AE significantly decreased elevated triglyceride levels in hyperlipidemic rats.

Serum HDL-Cholesterol

High cholesterol diet rats exhibited significantly lower HDL-C (Fig 3) levels as compared to normal control rats. Treatment with ME and AE significantly increased HDL-C levels as compared to high cholesterol diet rats.

Serum LDL

High cholesterol diet rats exhibited significantly higher LDL (Fig 4) levels as compared to normal control rats. Treatment with ME and AE extract significantly lowered levels of LDL as compared to high cholesterol diet rats.

Serum VLDL

High cholesterol diet rats exhibited significantly higher VLDL (Fig 5) levels as compared to normal control rats. Treatment with ME and AE significantly lowered levels of VLDL as compared to high cholesterol diet rats.

Atherogenic index and HDL-ratio

High cholesterol diet rats exhibited significantly higher atherogenic index (Fig 6) and lower the HDL-ratio as compared to control rats. Treatment with ME and AE significantly lowered the atherogenic index (Fig 7) and increased HDL-ratio.

Each bar in figure represents Mean \pm S.E.M. number of animals in each group = 6. R1 = control, R2 = high cholesterol diet control, R3 = high cholesterol diet treated with Petroleum ether extract of *Cedrela toona* Roxb. (1gm/kg, p.o.), R4 = high cholesterol diet treated with Chloroform extract of *Cedrela toona* Roxb. (1gm/kg, p.o.), R5 = high cholesterol diet treated with Methanol extract of *Cedrela toona* Roxb. (1gm/kg, p.o.), R6 = high cholesterol diet treated with Aqueous extract of *Cedrela toona* Roxb. (1gm/kg, p.o.) * significantly different from control, ** significantly different from high cholesterol diet control rats, $p < 0.05$.

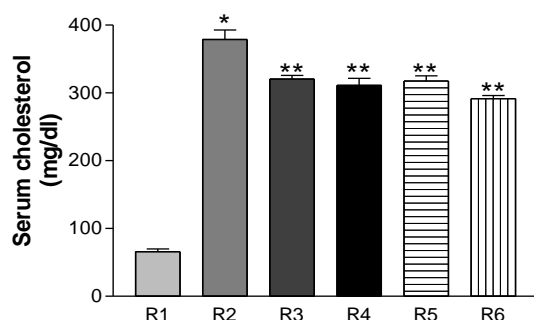


Figure 1: Effect of Various Extracts of *Cedrela toona* Roxb. On Serum Cholesterol levels

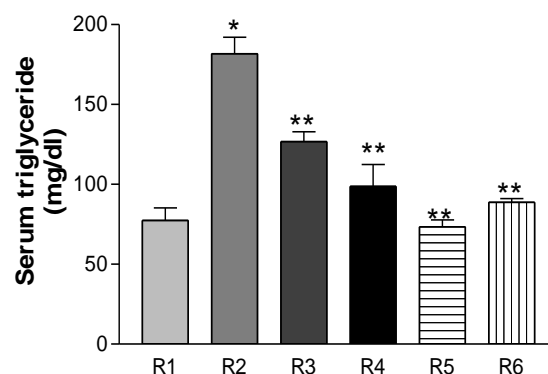


Figure 2: Effect of Various Extracts of *Cedrela toona* Roxb. On Serum Triglyceride levels

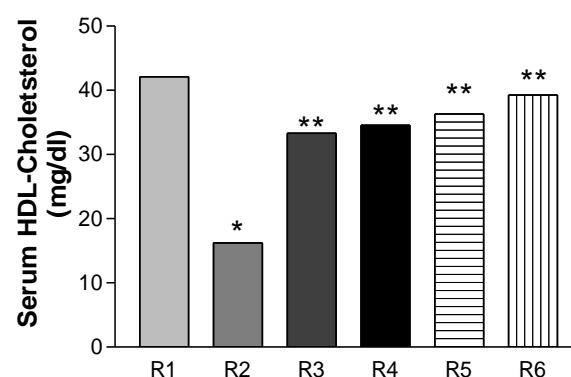


Figure 3: Effect of Various Extracts of *Cedrela toona* Roxb. On Serum HDL Cholesterol levels

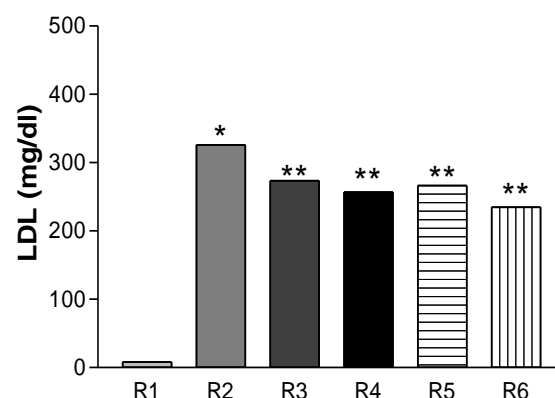


Figure 4: Effect of Various Extracts of *Cedrela toona* Roxb. On Serum LDL levels

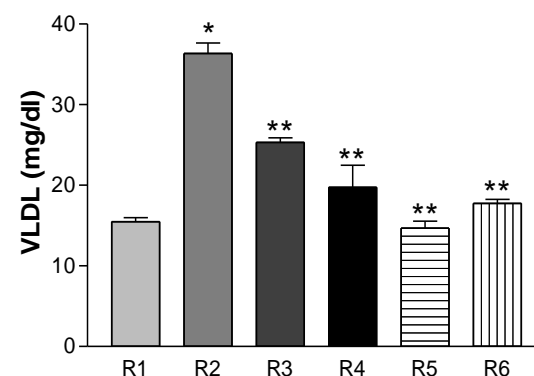


Figure 5: Effect Various Extracts of *Cedrela toona* Roxb. On Serum VLDL levels

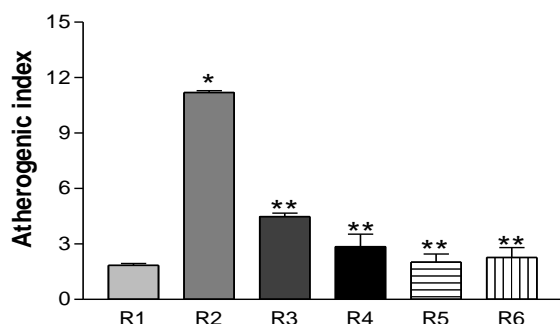


Figure 6: Effect of Various Extracts of *Cedrela toona* Roxb. On Atherogenic index

CONCLUSION

The present study suggested that the methanolic extract of *Cedrela toona* fruit possesses antihyperlipidaemic activity and therefore further studies can be taken up for drug discovery.

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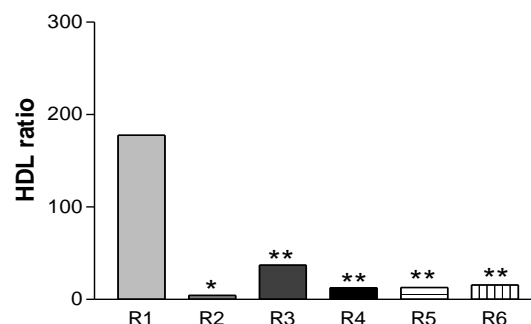


Figure 7: Effect of Various Extracts of *Cedrela toona* Roxb. On HDL Ratio

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