EVALUATION OF ANTIHYPERLIPIDAEMIC EFFECT OF CEDRELA TOONA ROXB. FRUITS

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INTRODUCTION

Litreture 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 – tuffs; petiole 4-11 cm long, petiolules 5-12 mm long. Peniciles 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, antidiysentric, antiperiodic.5 Flowers are emmenagogue, leaf is analgesic, aphrodisiac, antihemorrhagic, and good for scabias and expectorant (Yunani).6,7

Phytochemical studies reported the presence of Credrelone , isolated from the benzene extract of the heartwood of the Cedrela toona Roxb.8,9 sesquiterpenes, cycloartane stigmasterol, campesterol, apotirucallene, tirucallene, catechin, proanthocynidin, leucoanthocyanidin, toonacin, 6-acetoxy toonacin, toonacilin, geranyl geraniol, δ-cadinene, calamenene, α-calamone, siberin, deoxycedrelone.9 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)-melatiatriene-2,7,21-trione], along with product 4[14β,15β-epoxy-6,23-dihydroxy-1,5,20(22)-melatiatriene-2,7,21-trione].10,11 12α-hydroxystigmast-4-en-3-one: a new bioactive steroid isolated from the petroleum ether extract of Toona ciliate (Meliaceae) along with the two known steroid and three C- methyl coumarins.12 5-methylcoumarins isolated from the dried and powdered stem bark of Toona ciliate, extracted successively with light petroleum ether (40-60º), dichloromethane and methanol in soxhlet apparatus.13 Limonoids i.e.Toonaciliatins were reported from leaves and stem of Toona ciliate.14 Siderin, a natural coumarin was isolated from the methanolic extract of the leaves of Toona ciliate with the help of column chromatography.15 Toonafilin, a tetranortriterpenoid Blactone isolated from the ether extract of leaves of Toona ciliate. Polyynes isolated from the ethylacetate extract of the leaves of Toona ciliate. Seven new compounds were isolated from the petrol and chloroform extract of the trees of Toona ciliata, and there structure were identified as 3-Acetoxy 17-furan-3-yl-1-hydroxy-1,4,4,10,13-penta-methyl-12-oxo-tetradecahydro-16,20-dioxygenocyclopenta[14,15]cyclooctatetrahydrophenanthrene-7-carboxylic acid methyl ester, beta sitosterol, stigmasterol, n-C35H72, palmitic acid, n-C20H42,3-(3-Propyl-[1,1,3,1-tercyclohexan-3-yl)-propan-1-ol.17 9,10-dihydrofenanthenes isolated from the dichloromethane extract of the root of Toona ciliate.19 One new limonoid, toonaciliatone A, and one new tirucallane type triterpenoid, toonaciliatone A, along with three known compounds, methyl – 3b-acetoxy-1-oxomelic-14(15)-enate, perforin A, and cholest-14-ene-3,7,24,25-tetrol-21,23-epoxy-21-methoxy-4,8-trimethyl-3-(3-methyl-2-butenoate), were isolated from the leaves of Toona ciliata.19,20

Plant also possess antioxidant,21,22 Antiiulcer,23,24 Analgesic,25 Antifungal,26 Antimicrobial,27,28 Anti feedant, Anti tumor activity and cytotoxicity.29 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.

**Animals**31

Swiss albino/Sprague Dawely female rats weighing 150-200-gm were acclimatized to the experimental room having temperature 23 ± 2°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 Extracts**34

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° - 80°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 was 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).
Details of Biochemical Parameters Used

Cholesterol

Principle

\[
\begin{align*}
\text{Cholesterol Ester} + O_2 & \rightarrow \text{Cholesterol} + \text{Fatty acids} \\
\text{Cholesterol} + O_2 & \rightarrow \text{Dehydroacetone phosphate} + H_2O_2
\end{align*}
\]

\[
2H_2O_2 + \text{Phenol} + 4\text{-aminoantipyrine} \rightarrow \text{Red quinone} + 4H_2O
\]

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

\[
\begin{align*}
\text{Triglycerides} + H_2O & \rightarrow \text{Glycerol} + \text{free fatty acids} \\
\text{Glycerol} + ATP & \rightarrow \text{Glycerol-3-Phosphate} + \text{ADP} \\
\text{Glycerol-3-Phosphate} + O_2 & \rightarrow \text{Dehydroacetone phosphate} + H_2O_2
\end{align*}
\]

\[
2H_2O_2 + 4\text{-aminoantipyrine} + \text{ADPS} \rightarrow \text{Red quinone} + 4H_2O
\]

\[
\text{GPO} = \text{Glycerol-3-Phosphate Oxidase}
\]

\[
\text{ADPS} = \text{N-Ethyl-N-Sulfopropyl-n-anisidine}
\]

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 i.e. enzyme/chromogen mixture. They were incubated at 37°C for min and absorbance was read against blank at 546nm.

Calculation

\[
\text{Serum triglyceride (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of STD}} \times 200
\]
HDL-Cholesterol

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 x g 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:

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

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

\[
\text{HDL ratio} = \frac{\text{HDL-cholesterol x 100}}{\text{Total serum cholesterol} - \text{HDL-C}}
\]

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

Statistical Analysis

Results are presented as mean ± 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 ≤ 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±6.57 | 17.49±0.49 | 215.72±70.51 | 2.08±0.10 |
| 2      | Control | 447.39±21.66 | 36.35±1.29 | 4.42±0.35 | 8.57±0.09 |
| 3      | PE     | 373.70±25.86 | 25.33±0.54 | 7.15±0.47 | 4.48±0.18 |
| 4      | CE     | 256.83±5.53 | 19.74±2.73 | 12.50±2.43 | 2.85±0.67 |
| 5      | ME     | 266.50±4.98 | 14.67±0.88 | 12.91±2.43 | 2.02±0.43 |
| 6      | AE     | 234.62±0.15 | 17.74±0.49 | 15.58±2.16 | 2.26±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±1.90 | 87.44±2.45 | 42.12±1.20 |
| 2      | Control | 378.73±5.00 | 181.80±6.47 | 21.23±0.91 |
| 3      | PE     | 320.51±6.58 | 126.67±2.71 | 33.31±1.96 |
| 4      | CE     | 311.13±10.28 | 98.71±3.65 | 34.56±2.01 |
| 5      | ME     | 317.47±7.85 | 73.33±4.43 | 36.29±1.98 |
| 6      | AE     | 291.64±4.56 | 88.72±2.45 | 39.28±4.21 |
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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 extract significantly lowered 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 extract 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 extract 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 extract significantly lowered the atherogenic index (Fig 7) and increased HDL-ratio.

Each bar in figure represents Mean ± 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.

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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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