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, petiolum 5-12 mm long. Peticles 20-40 cm long. Petals 5-6 mm long, white. Capsule ellipsoid, 10-20 mm long, 6-8 mm in diameter; seeds winged at both ends1,2,3,4. Traditionally the bark is astringent, antidysentric, antispasmodic, antitussive1. Flowers are emmenagogue, leaf is cardiotonic, aphrodisiac, anthelmentic; good for scabies and expectorant (Yunani)5,6.  

Phytochemical studies reported the presence of Cedrelone *, isolated from the benzene extract of the heartwood of the Cedrela toona Roxb.5,6,7 sesquiterpene, cycloartenol stigmasterol, campesterol, apotirucullene, tirucallene, catechin, proanthocynidin ,leucoanthocynidin, toonacin, 6-acetoxy toonacin, toonacid, geranyl geraniol, δ-cadinene, calamenene, α-calicorene, siderin, deoxycedrelone7. Cedrelone, isolated from the benzene extract of heartwood of Toona ciliata, on photooxidation yield: 3[14β,15β,22β,23β-di-epoxy-6-hydroxy-6-hydroxy-1,5,20(22)- meliatriene-2,7,21-trione], along with product 4[14β,15β-epoxy-6,23-di-hydroxy- 1,5,20(22)-meliatriene-2,7,21-trione]7,8. 12α- hydroxyxstigmat-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 coumarins9. 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 apparatus10,11. Limonoids i.e.Toonaciliatins were reported from leaves and stem of Toona ciliata12. Siderin , a natural coumarin was isolated from the methanolic extract of the leaves of Toona ciliata with the help of column chromatography13. Toonafolin , a tetrnortrerpenoid Blactone isolated from the ether extract of leaves of Toona ciliata. Polyynes isolated from the ethylacetate extract of the leaves of Toona ciliata14. 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-dioxacyclopenta[14,15]cyclopenta[alpha]phenanthrene-7-carboxylic acid methyl ester, beta sitosterol, stigmasterol, n-C35H72, palmitinic acid, n-C20H42,3-(3-Propyl-1,1,3,1-tercyclohexan-3-yl)-propan-1-ol15. 9,10-dihydrophenanthenes isolated from the dichloromethane extract of the root of Toona ciliata16. One new limonoid, toonaciliatone A, and one new tircullane type triterpenoid, toonaciliatine A along with three known compounds, methyl – 3b-acetoxy-1-oxomelic-15-enate, perforin A, and cholest-14-en-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 ciliata17,18.  

Plant also possess antioxidant21,22, Antiulcer23,24, Analgesic25, Antifungal26, Antimicrobial27,28, 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 hyperlipidemic 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 standards.

**Animals**
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**
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**
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-I**: 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**
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

- Cholesterol esterase
  \[\text{Cholesterol ester} + \text{O}_2 \rightarrow \text{Cholesterol} + \text{Fatty acids}\]
- Cholesterol oxidase peroxidase
  \[\text{Cholesterol} + \text{O}_2 \rightarrow \text{Dehydroacetone phosphate} + \text{H}_2\text{O}_2\]

\[2\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{-aminoantipyrine} \rightarrow \text{Red quinone} + 4\text{H}_2\text{O}\]

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

- Lipoprotein lipase
  \[\text{Triglycerides} + \text{H}_2\text{O} \rightarrow \text{Glycerol} + \text{free fatty acids}\]
- Glycerol kinase
  \[\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-Phosphate} + \text{ADP}\]
- Peroxidase
  \[\text{Glycerol-3-Phosphate} + \text{O}_2 \rightarrow \text{Dehydroacetone phosphate} + \text{H}_2\text{O}_2\]

\[2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{ADPS} \rightarrow \text{Red quinone} + 4\text{H}_2\text{O}\]

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

ADPS= N-Ethyl-N-Sulfopropyl-α-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 fo 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

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

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

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

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

\[
\text{HDL ratio = } \frac{\text{HDL-cholesterol x 100}}{\frac{\text{Total serum cholesterol - HDL-C}}{5}}
\]

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

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

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

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

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