ANTIHYPERLIPIDEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF LAWSONIA INERMIS L. ROOT IN TRITON WR-1339 INDUCED HYPERLIPIDEMIC RAT

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ABSTRACT

Lawsonia inermis L. (Lythraceae), popularly known as henna, is a small tree or shrub. Its root has been used traditionally in Ayurveda for the treatment of hyperlipidemia. To our best knowledge no scientific study has been conducted for this effect. Therefore the present study was aimed to screen the antihyperlipidemic activity of hydroalcoholic extract of Lawsonia inermis L. root in Triton WR-1339 induced hyperlipidemic rat. Overnight fasted Wistar rats were randomly divided into normal control group (2% tween 80, p.o), positive control group (Triton WR-1339, 200 mg/kg, i.p), standard drug treated group (fenofibrate 65 mg/kg, p.o) and Lawsonia inermis extract treated group (200 & 400 mg/kg, p.o). After 24 hr. of treatment serum lipid profile was estimated. The extract significantly decreased serum TC, TG, VLDL, & LDL and increased serum HDL level. These results suggest that hydroalcoholic root extract of L. inermis has antihyperlipidemic activity and this validates its use in traditional Ayurvedic medicine. Preliminary phytochemical screening revealed the presence of carbohydrates, steroids, terpenoids, flavanoids, saponins and coumarins which are responsible for the effects. Further studies are needed to establish a clear mode of action.

KEY WORDS: Lawsonia inermis L. root, hyperlipidemia, Triton WR-1339

1. INTRODUCTION

It is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. Alteration and/ or abnormality in the metabolism of lipid and lipoproteins is a very common condition that taken place within(happens) in general
population, and is considered as one of the main risk factor in the incidence of cardiovascular disease due to their influence on atherosclerosis.\textsuperscript{[1]} The current antihyperlipidemic therapy includes HMG-CoA reductase inhibitors, bile acid sequestrants, fibric acid derivatives and nicotinic acid.\textsuperscript{[2]} These modern medicines have some side effects like hepatotoxicity, myopathy, gastrointestinal disturbances.\textsuperscript{[3]} Plant medicines are advantageous as being safer and less damaging to the human body than the synthetic drugs. Scientific studies on indigenous remedies resulted in the production of many therapeutic agents used in modern, conventional medicine.

In Ayurvedic traditional medicine, a decoction prepared from root of \textit{Lawsonia inermis} L. (Lythraceae), popularly known as henna, administered orally for the treatment of hyperlipidemia. \textit{Lawsonia inermis} L. is a small tree or shrub. It is a very useful medicinal plant found in almost all parts of the world. Its stem bark, leaves, roots, flowers and seeds have been used in traditional medicine. The leaves have anti-inflammatory, analgesic, antipyretic\textsuperscript{[4]}, antidiabetic\textsuperscript{[5]}, antiurolithiatic\textsuperscript{[6]} and diuretic activity.\textsuperscript{[7]} In traditional medicine its bark is used for burns and jaundice.\textsuperscript{[8]} Abortifacient,\textsuperscript{[9]} anticancer\textsuperscript{[10]} and hepatoprotective activities\textsuperscript{[11]} of root of \textit{L. inermis} was already reported. But there is no scientific report for antihyperlipidemic activity. So this study was aimed to screen antihyperlipidemic activity of hydroalcoholic extract of \textit{Lawsonia inermis} L. root in Triton WR 1339 induced hyperlipidemic rats.

\section*{2. MATERIALS AND METHODS}

\subsection*{2.1. Plant materials}

\textbf{1.1. Selection, collection and identification of plant material}

The plant material was collected in November-December from Kottayam district in Kerala, India. The plant were taxonomically identified and authenticated by Dr. George Joseph, head of Botany department, Sacred Heart College, Thevara, Ernakulum, Kerala.

\textbf{1.2. Preparation of 80\% ethanolic extract of \textit{Lawsonia inermis} root.}

The collected roots were washed with water, cut into small pieces and dried under the shade. The dried plant material was milled to a fine powder using the commercial laboratory blender. The dried powder was extracted in a Soxhlet extractor with hydroethanol (80:20\%). The extraction was continued for 72 hour or until the solvent in the thimble was cleared. Air dried the extract and was then stored in a desiccator & used for further investigations. Fresh solutions of \textit{L. inermis} were prepared on each day of the experiment by
reconstituting a weighed quantity of the crude extract with 2% tween 80. The percentage yield was 4.7%

1.3. Preliminary phytochemical screening
Phytochemicals are chemical compounds that occur naturally in plants. The presence of various phytoconstituents like alkaloids, carbohydrates, steroids, triterpenoids, cardiac glycosides, flavonoids, saponins and tannins were determined by the standard qualitative methods.\[12,13\]

2.2. Drugs and chemicals
Fenofibrate 65mg/kg (Cipla), Hydroalcoholic extract of L. inermis 200 & 400 mg/kg, Triton WR-1339(200mg/kg, Sigma–Aldrich, USA), total cholesterol, triglyceride, HDL diagnostic kit (M/S Excel diagnostic, Pvt. Ltd, Hyderabad. Triton WR -1339 was used to induce hyperlipidemia. Plant extract and triton was suspended in 2% tween 80 which was used as vehicle. The standard drug was dispersed in distilled water containing vehicle. All drugs were given orally in a volume of 0.5ml /100g body weight of rat using an oral feeding tube. Fresh drug solutions were prepared on each day of the experiment.

2.3. Experimental animals & exposure conditions
The animal albino Wistar rats were purchased from Animal house, Gov. Veterinary College Mannuthy. The animals were fed with rat feed and water ad libitum. They were housed in clean poly propylene cages, under identical conditions of food, water and temperature. They were exposed to 12 hours, light-dark cycle and the relative humidity was in the range of 61-76% and temperature range was 15-25°C. All procedures were performed according to CPCSEA guidelines after proper approval from the Institutional Animal Ethics Committee (IAEC, proposal no. SJCP/IAEC/04/2014), St. Joseph’s College of Pharmacy, Cherthala.

2.4. Selection of dose of the extract
Dose of the extract was selected by acute oral toxicity studies. Manjula et al; (2012) and other researchers/literatures reported that hydroalcoholic root extract of L. inermis was nontoxic up to dose 2000 mg/kg body weight and extract did not produce any mortality.\[14\] Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further studies.
2.5. Antihyperlipidemic activity - Triton-induced hyperlipidemia

The systemic administration of the surfactant, Triton to mice or rats results in a biphasic elevation of plasma cholesterol and triglycerides\textsuperscript{[15,16]} (Frantz and Hinkelman 1955; Garattini et al.)

**Procedure**

Male Wistar rats weighing 180-200 g were used for the study. The hyperlipidemia was induced by the intraperitoneal injection of Triton WR-1339 (200 mg/kg). Animals were starved for 18h and divided into five groups of six rats each. Group I: Administered vehicle (2% tween 80) p. o and served as normal control, Group II: Administered Triton WR 1339; i. p and served as hyperlipidemic control, Group III: Administered fenofibrate (65 mg/kg), p. o. and served as standard, Group IV: Administered \textit{L. inermis} (200mg/kg), p. o. Group V: Administered \textit{L. inermis} (400mg/kg), p. o. All the treatments were given orally, immediately after triton injection except normal control group. After 24 hour blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500 rpm. The serum samples were collected and lipid profiles were analysed.

**Estimation of lipid profile:** Serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C) were analysed by using diagnostic kit. Very Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) levels were calculated using Friedewald formula.

\[ \text{Serum VLDL-C} = \frac{\text{TRIGLYCERIDE}}{5} \]

\[ \text{Serum LDL-C} = \text{Total Cholesterol} - (\text{HDL-C} + \text{VLDL-C}) \]

2.6. **Statistical evaluation**

The results were expressed as mean ± SEM. Statistical analysis of all the data obtained were evaluated using one-way ANOVA followed by Dunnett’s post -hoc multiple comparison test with SPSS Program ;Version 20. All the results were also expressed as graph by Graph Pad Prism software (v.5). P values < 0.05 were considered as statistically significant.

3. **RESULTS**

3.1. **Phytochemical screening of plant extract:** The preliminary phytochemical studies of extract revealed the presence of carbohydrate, sterols, terpenoids, flavanoids, saponins, tannins &coumarins and absence of alkaloids. The obtained results were shown in table 1
Table 1: Preliminary phytochemical screening of *L. inermis*

<table>
<thead>
<tr>
<th>CONSTITUENTS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Sterols &amp; terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>tannins</td>
<td>+</td>
</tr>
</tbody>
</table>

3.2. Antihyperlipidemic activity – Triton induced hyperlipidemia

The serum lipid level of normal control group, hyperlipidemic control group and drug treated groups are shown in Table 2. The statistical result showed that the triton treated group significantly (P<0.001) increased the serum TC, TG, VLDL & LDL levels and significantly decreased (P<0.001) HDL-C level when compared to normal (vehicle control) group. In comparison with the hyperlipidemic control group, standard drug fenofibrate treated group significantly reduced (P<0.001) serum TC, TG, VLDL & LDL level and significantly increased (P<0.001) serum HDL level. *Lawsonia inermis* extract 200 & 400 mg/kg significantly decreased (P<0.01) serum TC, TG, VLDL & LDL. Extract with dose 200 & 400 mg/kg increased serum HDL level significantly at P<0.05 and P<0.01 respectively. The effect of standard and extract treated groups on serum TC and TG levels are shown in fig. 1 and its effect on serum VLDL & LDL are shown in fig. 2. The effect of drug treated groups on serum HDL level are shown in fig. 3.

Table 10: Effect of hydro alcoholic extract of *Lawsonia inermis* root (200 & 400 mg/kg, p.o) and fenofibrate (65 mg/kg, p.o) on serum lipid level in triton induced hyperlipidemic model

<table>
<thead>
<tr>
<th>TREATMENT GROUPS</th>
<th>SERUM LIPID PARAMETERS (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TC</td>
</tr>
<tr>
<td>I. NORMAL CONTROL (0.2% tween 80, p.o)</td>
<td>84.08±1.14</td>
</tr>
<tr>
<td>II. HYPERLIPIDEMIC CONTROL (Triton 200 mg/kg, i.p)</td>
<td>161.67±0.67***</td>
</tr>
<tr>
<td>III. STANDARD (Triton + fenofibrate 65mg/kg)</td>
<td>104.99±1.40***</td>
</tr>
<tr>
<td>IV. TEST- 200 (Triton + <em>L. inermis</em> extract 200 mg/kg)</td>
<td>128.79±3.89**</td>
</tr>
</tbody>
</table>
All values are expressed as mean ± SEM for six animals in each group using one-way ANOVA followed by Dunnett ‘t’ test. # P < 0.05, ## P< 0.01, ### P< 0.001 – compared to normal control. *P< 0.05, ** P< 0.01, *** P< 0.001 – compared to hyperlipidemic control.

Fig 1: Effect of hydro alcoholic extract of *Lawsonia inermis* L. root (200 & 400 mg/kg, p.o) and fenofibrate (65 mg/kg, p. o) on serum TC& TG level in triton induced hyperlipidemic model.
Normal Control  Triton Control  Fenofibrate  L. inermis  L. inermis
0  20  40  60  
***  **  **  
TREATMENT
  65 mg/kg  200 mg/kg  200 mg/kg  400 mg/kg  
###
SERUM VLDL LEVEL (mg/dl)

# P < 0.05, ## P < 0.01, ### P < 0.001 – compared to normal control. *P < 0.05, ** P < 0.01, *** P < 0.001 – compared to hyperlipidemic control.

Fig 2: Effect of hydro alcoholic extract of *Lawsonia inermis* L. root (200 & 400 mg/kg,p. o) and fenofibrate (65 mg/kg, p. o) on serum VLDL & LDL level in triton induced hyperlipidemic model

Normal Control  Triton Control  Fenofibrate  L. inermis  L. inermis
0  10  20  30  40  50  
***  *  **  
TREATMENT
  65 mg/kg  200 mg/kg  200 mg/kg  400 mg/kg  
###
SERUM HDL LEVEL (mg/dl)

# P < 0.05, ## P < 0.01, ### P < 0.001 – compared to normal control. *P < 0.05, ** P < 0.01, *** P < 0.001 – compared to hyperlipidemic control.

Fig.3: Effect of hydro alcoholic extract of *Lawsonia inermis* L. root (200 & 400 mg/kg,p. o) and fenofibrate (65 mg/kg, p. o) on serum HDL level in triton induced hyperlipidemic model
DISCUSSION AND CONCLUSION

Triton WR-1339 acts as a surfactant and induces an acute hyperlipidemia in many animals. Intraperitoneal administration of 200 mg/kg of triton WR-1339 significantly increased the serum TC, TG, VLDL, LDL level and decreased the serum HDL lipoproteins level of normal rats. In this study we observed that the hydroalcoholic extract of *L. inermis* treated groups reverse these effects i.e., it showed a significant decrease in TC, TG, VLDL, LDL and significant increase in HDL level when compared to triton induced group.

Triton induces hyperlipidemia by biphasic mechanism. Serum cholesterol levels increase sharply 2–3 times after 24 h (phase I). The hypercholesterolemia decreases nearly to control levels within the next 24 h (phase II). Drugs interfering with cholesterol biosynthesis and uptake were shown to be active in phase I (synthetic phase) while the drugs interfering with cholesterol excretion and metabolism were active in phase II (excretory phase). The mechanism of hypolipidemic activity of *L. inermis* may be due to inhibition of cholesterol synthesis and bile acid excretion. The results showed the lowering of TC level by *L. inermis* 200 & 400mg/kg was associated with a significant decrease of LDL level and significant increase of HDL level. Lecithin Cholesterol Acyl Transferase (LCAT) plays a key role in incorporating free cholesterol into HDL and transferring back to VLDL or IDL which is taken back by the liver cells. Lowering of LDL level may be due to an increase in LDL receptors. Depletion of intracellular cholesterol causes the cell to increase the number of specific cell-surface LDL receptors that can bind and internalize circulating LDLs. Thus, the end result is a reduction in plasma cholesterol, both by lowered cholesterol synthesis and by increased catabolism of LDL. *L. inermis* 200 & 400 mg/kg significantly decreased TG level. This could be due to the enhanced catabolism of triglyceride. An increased stimulation of lipolytic activity of plasma lipoprotein lipase (LPL) causes the catabolic metabolism of triglycerides.

The lipid lowering effect of *L. inermis* may probably be due to presence of secondary metabolites in plant. The previous studies and the phytochemical screening of present study showed the presence of sterols, triterpenoids, saponins, flavanoids and tannins. Flavanoids augment the activity of LCAT. From these evidences we can say that *Lawsonia inermis* root extract have antihyperlipidemic activity. *L. inermis* showed an increase in HDL level which suggests the further exploration on the other extracts of *Lawsonia inermis* may be worth. Further studies on drug metabolism and excretion are needed to elucidate a clear mechanism.
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REFERENCES