Antihyperlipidemic Activity of the Methanolic extract from the Stems of *Tinospora cordifolia* on Sprague dawley rats

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ABSTRACT

The work presented herein is about the cholesterol lowering properties of methanolic extracts of *Tinospora cordifolia* stems in hyperlipidemic Sprague dawley rats. Hyperlipidemia was induced in rats by once a day administration of cholesterol using olive oil as vehicle at 500 mg kg⁻¹ b.w. orally. The methanolic extracts of the stem of *T. cordifolia* at 200 mg kg⁻¹ b.w. and 400 mg kg⁻¹ b.w. were evaluated for their possible antihyperlipidemic activity by simultaneous administration along with cholesterol in olive oil orally for 30 days. There was a significant increase in total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein and very low density lipoproteins in all the cholesterol fed groups, however the groups tested with methanolic extracts exhibited a significant reduction in their levels. The reduction was compared with standard atorvastatin and it was significant. The high density lipoproteins were relatively not affected and the reduction by the methanolic extract at 200 mg kg⁻¹ b.w. dose was insignificant, however at 400 mg kg⁻¹ dose there was a significant reduction in their levels. The plant needs a thorough phytochemical investigation to find the possible constituents responsible for the activity and to derive the exact mechanism of action.

Key words: Atherosclerosis, *Tinospora cordifolia*, low density lipoprotein, high density lipoprotein, hypercholesterolemia.

INTRODUCTION

Atherosclerosis and cardiovascular diseases are the leading cause of mortality and morbidity worldwide [1]. Hypercholesterolemia and hyperlipidemia contributes significantly in the manifestation and development of coronary heart diseases. The level of cholesterol decides the risk of developing cardiovascular disease, higher levels of cholesterol then greater will be the risk of cardiovascular diseases. Cholesterol is an important integral part of new cells, insulating
material of nerves and also a raw material in the production of hormones. Liver is associated with the cholesterol metabolism, as long as the levels of cholesterol are normal the risk is less, but because of unhealthy food habits the levels may greatly change and increase the risk of cardiovascular disease. The contribution of diet for atherosclerosis is critical and hence food habits should be altered in such a way to intervene high cholesterol levels especially LDL cholesterol, which is considered as the responsible cholesterol for many cardiac failures [2]. Among different types of cholesterols like low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL), LDL is considered as bad cholesterol which can buildup in the walls of arteries and form hard plaque thereby increasing the risk, whereas the HDL is generally known as good cholesterol as it is less associated with cardiac disease. It is evident that obesity, hypertension, hypercholesterolemia and diabetes mellitus are interrelated with the outcome of atherosclerosis and hence need to be checked at the childhood to predict possible cardiac complications at the later age. The lipid content in the diet, genetic factor, age, sedentary life style, smoking and stress are the major other causes of atherosclerosis.

At present atherosclerosis is treated with popular statins and there is an increasing trend in the prescriptions of the physicians to treat hyperlipidemia using statins. There is a growing pressure on the dispensing pharmacist to give these drugs as over the counter medicine. The current antihyperlipidemic drugs though effective in minimizing the levels of cholesterol has lot of adverse effects associated and there is a need for alternative agents to control atherosclerosis and with minimum side effects. Statins are synthetic and studies show that they have serious side effects like liver and muscle damage. Further the adverse effects like gastrointestinal disturbances causing nausea, dyspepsia, abdominal pain, skin related side effects, cataract, depression, peripheral neuropathy and some autoimmune disorders were also reported [3]. There is enough evidence on the effect of statins on erectile dysfunction [4, 5], renal effects [6] and also in cancer [7] as well. Natural products always found to be the reliable source for several ailments, their popularity and contribution is undoubtedly worthless. Natural plants have a dramatic cholesterol lowering properties without any side effects which are normally associated with synthetic drugs.

*Tinospora cordifolia* (Menispermaceae) is the herb commonly used in Indian system of medicine and is found growing throughout the plains of India, known as guduchi. The species is well known in the folklore medicine as antidiabetic, antipyretic, antiulcer, antioxidant, hepatoprotective, immunomodulatory and also for its hypolipidemic properties [8-10]. But much of the pharmacological evidence is lacking for these activities.

The plant is reported to have alkaloids like tinosporine, palmitine and glycosides like tinocordiside, tinocordifolioside and also some terpenoids [11,12]. Earlier on *T. cordifolia* the pharmacological screening has been done for anti-inflammatory, immunomodulatory, anticancer, anti diabetic, anti ulcer, anti rheumatic activities. However antihyperlipidemic activity was not done on aerial parts and was felt appropriate to evaluate on the stems. Hence in the present study the antihyperlipidemic activity of the methanolic extract of stem was studied for the possible activity on high cholesterol fed rats.
MATERIALS AND METHODS

Plant material
The fresh stems of Tinospora cordifolia were collected from in and around Tirupati, Andhra Pradesh, India, authenticated by Dr. M. Venkaiah, taxonomist, India, a voucher specimen (DTP/TC/3/2010) was deposited in the institution herbarium and they were shade dried. The dried material was coarsely powdered in an electrical mill and 500 g of the material was used for successive solvent extraction using petroleum ether, chloroform and methanol in Soxhlet apparatus. The corresponding methanolic residue (19.23 g) obtained was used for the present study.

Enzymatic kits and chemicals
Cholesterol, triglycerides, HDL, LDL and VLDL were estimated by using different enzymatic kits, Cholesterol kit (Cat. No. OSR 6516), triglyceride kit (Cat. No. OSR 6133) were purchased from Olympus, Hamburg, Germany and LDL, HDL and VLDL kit (Cat.No. E2HL100, EnzyChrom™) was purchased from BioAssay systems, Hayward, USA, using Olympus clinical chemistry analyser (AU 400) Hamburg, Germany. Cholesterol, was purchased from Loba Chemie, Mumbai, Olive oil was procured from the local market. Atorvastatin was a gift sample from M/s. Micro labs, Hosur, India.

Animals and dietary treatment
Adult Sprague Dawley rats of either sex, weighing 150–200 g were purchased from Ghosh enterprises, Kolkata, India. They were housed at a room temperature of 25 ± 2 °C, relative humidity of 75 ± 5% and 12 h dark–light cycle and standard basal diet was provided in the form of pellets supplied by M/s. Rayon Biotech, Hyderabad and water ad libitum was given throughout the study period of 30 days. The experimental protocol was approved by the Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) and the experiments were conducted in accordance with the principles prescribed for laboratory animal use.

The rats, randomly divided into 6 groups of six animals each (Group-I to VI), were fed differently in addition to the standard basal diet. Group-I was maintained as control (without any treatment), group-II was treated with the vehicle used (Olive oil). Hyperlipidemia was produced in group-III, IV, V and VI by feeding 500 mg kg⁻¹ body weight cholesterol orally [13], dispersed in Olive oil (10 mL kg⁻¹ b.w. per day) once a day for 30 days [14]. Simultaneously Methanolic residue was administered to group-IV and group-V at a dose of 200 mg kg⁻¹ b.w. per day and 400 mg kg⁻¹ b.w. per day respectively dispersed in olive oil orally. Group-VI was administered with standard antihyperlipidemic drug, atorvastatin at a dose of 30 mg kg⁻¹ b.w. per day [15].

Collection of blood
After 30 days of treatment, the rats in all the groups were fasted overnight and 1 mL of blood was withdrawn from each rat by puncturing the retro orbital plexus and blood was allowed to clot, centrifuged at 5000 rpm for 15 min., the supernatant layer was pipetted out and used for biochemical analysis.
Estimation of cholesterol, triglycerides, HDL, LDL and VLDL content in serum

The method for estimation of cholesterol in the present investigation is based on the principle described by Stadtman et al. [16] and Flegg et al. [17] and results were as given in Table 1. The triglyceride estimation is based on the procedure described by Hagen et al. [18]. The principle for the estimation of HDL, LDL and VLDL is by improved precipitation method as given in the enzyme kit supplied by BioAssay systems. The LDL was determined by the following Freidewald’s formula [19].

\[
LDL = \text{Total cholesterol} - \left( \frac{\text{Triglycerides}}{5} \right) - \text{HDL}
\]

Statistical analysis of the data

Statistical significance of the data was analyzed using one way analysis of variance (ANOVA). The values were expressed in mean ± S.D, the values of test at *p < 0.05, **p < 0.01 were considered to be statistically significant.

RESULTS AND DISCUSSION

In the present study it was observed that keeping the rats on high cholesterol diet for 30 days significantly increased the total cholesterol, triglycerides, HDL, VLDL and LDL levels in serum, as compared to normal control rats. The increase in these lipid levels is marginal in case of group-II which is treated with olive oil alone and it is insignificant compared to control group. Continuous administration of cholesterol to group-III, IV and V resulted in the elevation of serum cholesterol, the raise in cholesterol was significant. The serum cholesterol in group-VI was less than the normal control group and this may be due to the effect of atorvastatin. There was a significant reduction in the serum cholesterol levels in methanolic extract treated groups. The triglycerides of serum were significantly reduced by methanolic extracts at both the dose levels tested, the two dose levels of *T. cordifolia* methanolic extract reduced triglycerides when compared to treated control. These results support the influence of the extracts in reducing the triglyceride levels though not as that of standard drug. The raise in LDL was in correlation with the cholesterol raise and it is relatively higher than the other lipids. The extracts exhibited LDL reduction in serum, though not comparable to the reduction of atorvastatin but significant.

HDL levels were raised significantly in serum of group-III and in group-IV, HDL is considered as good cholesterol and their levels were relatively not reduced much by the extracts and which is essential in the prevention of atherosclerosis. The VLDL concentration in serum was significantly increased in group-III.

Atorvastatin is HMG-CoA reductase inhibitor and reduces cholesterol, LDL levels directly by interfering with the rate limiting step in cholesterol biosynthesis and by enhanced LDL receptors in liver. It also reduces the triglycerides, VLDL levels especially by their clearance, increased LDL receptor uptake or other lipoprotein receptors or VLDL delipidation [20]. This is in correlation with the results obtained in the group-VI. The extracts may not have the similar mechanism of action by interfering HMG-CoA reductase and may be due to the interference of cholesterol with residues at the absorption site in gastrointestinal tract since they were co-administered. The HDL levels were not affected much by the extracts, this is a beneficial effect.
as HDL is considered as good cholesterol as it reduces cholesterol in cells and risk associated with atherosclerosis. However a precise mechanism of action needs to be derived.

Thus cholesterol, triglycerides, HDL, LDL and VLDL levels showed a significant decline when high cholesterol diet was co-administered with the extracts in a dose dependent manner. Epidemiological and clinical studies have demonstrated a positive correlation between LDL concentration in serum and the incidence of coronary heart disease. This is well supported by the high cholesterol and LDL levels in serum of group-III, IV and V. In the present study, a significant decrease in triglycerides, cholesterol and LDL levels in methanolic extract treated groups was observed, but at the same time HDL levels were relatively not affected much. Overall the extracts could able to reduce lipids significantly in serum at both the test doses.

Table 1: Effect of methanolic extract from Tinospora cordifolia on lipid profile of hyperlipidemic rats (n=6)

<table>
<thead>
<tr>
<th>Parameter (mg dL⁻¹)</th>
<th>Group-I (mean±s.d.)</th>
<th>Group-II (mean±s.d.)</th>
<th>Group-III (mean±s.d.)</th>
<th>Group-IV (mean±s.d.)</th>
<th>Group-V (mean±s.d.)</th>
<th>Group-VI (mean±s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>38.27±5.56</td>
<td>42.25±6.4</td>
<td>188.07±12.48**</td>
<td>158.4±13.26**</td>
<td>135±12.66**</td>
<td>36.82±4.24</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>45.35±9.76</td>
<td>51.44±6.48</td>
<td>112.75±12.37*</td>
<td>86.95±11.48*</td>
<td>64.22±7.33*</td>
<td>32.45±3.2</td>
</tr>
<tr>
<td>LDL</td>
<td>17.77±2.47</td>
<td>18.64±2.12</td>
<td>152.33±15.86**</td>
<td>118.04±15.37**</td>
<td>89.33±13.45**</td>
<td>14.22±1.89</td>
</tr>
<tr>
<td>HDL</td>
<td>19.43±2.65</td>
<td>18.35±2.7</td>
<td>34.78±2.67*</td>
<td>30.56±5.65</td>
<td>27.15±5.78*</td>
<td>13.85±2.25</td>
</tr>
</tbody>
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*p < 0.05, **p < 0.01, all the groups were compared with control group-I and group-III

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Among the natural materials, medicinal plants hold promise in the discovery of new drugs. T. cordifolia is been used in ayurvedic formulations, its hypolipidemic activity suggests that the plant needs a thorough phytochemical investigation to find the possible constituents responsible for the activity and to derive the exact mechanism of action. However with careful adjustments in diet, physical activity, controlling weight and effective management of stress one can prevent occurrence of cardiovascular diseases.

REFERENCES


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