Hypolipidemic effect of *Azima tetracantha* on lipid profile markers of carbon tetrachloride induced liver toxicity in rats

Saleem K*, Nargis Begum T, Muhammad Ilyas MH

1Research Scholar, Department of Biochemistry, PRIST University, Tamil Nadu, India
2Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli-620 020, Tamil Nadu, India
3Department of Biotechnology, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India

**ABSTRACT**

This study was aimed at evaluating the hypolipidemic effects of ethanolic extract of *Azima tetracantha* Leaves against liver toxicity induced by carbon tetrachloride (CCl₄) in male albino Wistar rats and to compare the same with the reference drug silymarin. Six groups of rats with six rats in each group were used as the experimental subject. Animals were allocated into a control group and liver toxicity control group. The remaining four groups received in addition to CCl₄, silymarin (20 mg/kg/d) as a reference treatment and *Azima tetracantha* (100, 200 and 400 mg/kg/d). Once the experiment period was completed, the biomarkers of lipid profile, including total cholesterol, serum triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were evaluated. *Azima tetracantha* significantly decreased the serum lipid profile markers cholesterol, triglycerides, phospholipids, fatty acids, VLDL, LDL, and increased HDL. *Azima tetracantha* could be a promising protective agent against cholesterol through the improvement of liver function, modulation of CCl₄ by-products formation and thus has hypolipidemic potentials.

**INTRODUCTION**

Lipids, like phosphoglycerolipids, sphingolipids, and neutral lipids, are natural components of living membranes and lipoproteins that are circulating in the blood. These lipids have an integral role in multiple biological processes, including apoptosis, proliferation, inflammation, and differentiation (Mene et al., 1989; Ridgway, 2013). Any alterations in the highly controlled lipid homeostasis can cause hepatic diseases and injury. To understand the pathophysiology of such diseases, the characterization of the alteration of lipids must be of key focus. One of the key functions attributed to the liver is invariably the synthesis of lipoproteins. The close correlation between the composition of lipids in the liver and that in the plasma has been unraveled in a recent study (Kotronen et al., 2010). Hence in understanding the hepatic pathophysiology, plasma lipid profiling is of immense significance and is being in practice. It has been already used in identifying biomarkers for hepatocellular carcinoma (Patterson et al., 2011; Chen et al., 2013) liver phospholipidosis (Saito et al., 2014), and non-alcoholic fatty liver disease (Puri et al., 2009).

Carbon tetrachloride (CCl₄) is one of the best agents in inducing liver injury and can give a platform for its pathophysiological studies. (Boll et al., 2001). It has...
been notorious for causing harm to the liver, central nervous system, lungs, and adrenals, both in human and experimental animals (McGregor and Lang, 1996). CCl₄ undergoes biological transformation by microsomal P450 of the liver. This results in the production of the liver toxifying metabolite called trichloromethyl radical (CCl₃). This is later converted to a peroxy radicals in the presence of oxygen. These products might interact with the lipids of living membranes resulting in its peroxidation (Muriel, 1997). An imbalance between the production and mortification of lipids is caused by CCl₄ (Boll et al., 2001).

In view of this, this study was carried out to analyze the effect of Azima tetracantha ethanolic leaves extract administration, on the lipid profile in carbon tetrachloride-induced hyperlipidemic rats.

**MATERIALS AND METHODS**

**Animals**

Wistar strain albino rats (male) of, approximately 3-4 months (weighing around 140-160g) were the experimental subjects. They were bought from Sri Venkateswara Enterprises, Bangaluru, India in good health conditions. They were accommodated in plastic cages in which rice husk was used as bedding. The cages were well aerated and were retained under standard experimental conditions (Twelve hours light and dark cycle, Temperature 27±2°C) throughout during the experimental phases. They were nourished with standard pellet diet obtained from Gold Mohur, Mumbai, India and water ad libitum. The subjects were attuned for a period of 1 week before the experimental use. The experiment strictly followed the recommendations of the Ethical Committee (Ethical No: SAC/IAEC/BC/2016/Ph.D-005), which stands for supervising and controlling the experiments done on Animals (CPCSEA New Delhi, India).

**Chemicals**

Carbon tetrachloride (CCl₄), thiobarbituric acid (TBA), Nitro blue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), trichloroacetic acid (TCA), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (oxidized). Glutathione (reduced). Carbon tetrachloride and L-ascorbic acid were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were taken from Glaxo Laboratories situated in Mumbai, India, and Sisco Research Laboratories, Mumbai, India and were of analytical grade.

**Plant Material**

Garden fresh leaves of *A. tetracantha* were gathered from Melur, Thiruchirappalli District, Tamil Nadu, South India in January 2015. The specimen was authenticated and identified by Dr S. John Britto, Director, ‘The Rabinat Herbarium and center for molecular systematics’, St. Joseph’s college Trichy-Tamil Nadu. India. Its Specimen voucher (EP001) is stored at the Rabinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

**Preparation of Plant Extract**

The leaves of *Azima tetracantha* were thoroughly washed, cut and later desiccated in a hot-air oven at 37°C. The dried and desiccated material was ground to obtain fine dust, which was later used to extract the phytoconstituents. Two hundred and fifty grams (250g) of the powdered material was then extracted with hexane, chloroform, and ethanol in “Soxhlet Apparatus” for around 48 hours. Resultant was a semi-solid material; once alcohol was eliminated under minimized pressure. It was later stored in the refrigerator. The extracted material had polar as well as non-polar phytoconstituents in it. For our experiments, 100, 200 and 400mg/kg bodyweight of *Azima tetracantha* ethanolic leaves extract was used. Selection of the effectual dose was based on the results of dose-dependent experiments performed with the extract which was conducted in our laboratory.

**Experimental Design**

After recording the bodyweight of the animals, they were divided into 6 groups, each comprising 6 animals listed as follows.

**Group I – Normal Rats**

**Group II – Negative control -** Animals were administrated orally with CCl₄ (0.5 ml/150g of bw-v/v in olive oil) on 1st, 8th and 16th day.

**Group III** – Animals were administrated orally with CCl₄ (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant extract (100mg/ Kg bw) orally for a period of 21 days.

**Group IV** - Animals were administrated orally with CCl₄ (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant’s extract (200mg Kg bw) orally for a period of 21 days.

**Group V** - Animals were administrated orally with CCl₄ (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with selected plant’s extract (400mg/ Kg bw) orally for a period of 21 days.

**Group VI -** Animals were administrated orally with CCl₄ (0.5 ml/150 g of bw-v/v in olive oil on 1st, 8th and 16th day) and treated with Silymarin (20mg/ Kg bw) orally for a period of 21 days.

**Collection of Various Samples**
After completing the experimental duration, animals were killed by decapitation. Plasma was separated, and biochemical parameters were analyzed.

Biochemical parameters

Cholesterol in the serum and tissue was estimated by the method of Parekh and Jung (1970). Triglycerides in the serum and tissue were estimated by the method of Foster and Dunn (1973), Phospholipids was estimated by the Bartlett method (1959). Non-esterified fatty acids estimation was by the Falholt et al. method (1973). Serum HDL was estimated by Friedewald et al. method, (1972).

Statistical Analysis:

The mean ± SD for six rats in each group were expressed as numerical values. One way analysis of variance (ANOVA) was employed for determining statistically significant differences between mean values. It was later followed by individual comparison that was obtained by Duncan’s Multiple Range Test (DMRT) using the SPSS software for Windows Version 20.0 (IBM Corp. Armonk, New York, NY, USA). A value of p < 0.05 was contemplated to indicate a significant difference between groups.

RESULTS AND DISCUSSION

Effect of Azima tetracantha leaves extract lipid profile markers

CCl₄ showed a marked increase in serum total cholesterol, free fatty acids, phospholipids, LDL, and TGS levels, while a noticeable decrease in serum level of HDL compared to normal control groups. Treatment of rats with Azima tetracantha ethanolic extract of leaves or silymarin significantly reduced serum total cholesterol, phospholipids, free fatty acids, LDL, and TGS levels whereas the serum level of HDL was significantly increased as compared to the control. Azima tetracantha leaves extract restored cholesterol to normal levels, like silymarin restoring TGS to normal levels (Table 1)

Results of this investigation disclosed that CCl₄ increased serum levels of triglycerides, cholesterol, and LDL, while it produced a remarkable decrease in serum levels of HDL. Our observations add further evidence for the previous reports of (Andritoiu et al., 2014), that CCl₄ aggravates lipid profile by cellular oxidative stress. Mediated by the free radicals derived from CCl₄, hepatic lesions are produced. The more the oxidative stress is intensified, the more enhanced the influence of non-essential fatty acids to aggravate the serum levels of cholesterol and triglyceride.

CCl₄ induced fatty liver development involves predominant cellular mechanisms like lipid peroxidation and radical formation (Tribble et al., 1987). Accumulation of lipids in large volumes in the liver is symptomatic underlying a pathological condition and in chronic situations; these cells undergo fibrosis that headway to cirrhosis and thus resulting in impeded liver function (Murray et al., 1993). CCl₄ hastens fatty acids and triglycerides synthesis from acetate. CCl₄ could cause the movement of acetate into the liver cell, which results in elevated availability of the substrate (acetate) for its conversion to the above lipids. During CCl₄ toxicity, cholesterol synthesis is also found aggravated (Boll et al., 2001).

The hydrolysis of triglycerides and β-oxidation of fatty acids is found to ameliorating upon CCl₄ introduction. This intensifies fatty acids availability for esterification (Lieber, 2000). Moreover, it is reported that CCl₄ toxicity results in the accumulation of fat in the liver and kidney, which has been due to the translocation of the same from the peripheral adipose tissue. (Devarshi et al., 1986). Apolipoproteins synthesis is found to be inhibited by CCl₄ henceforth resulting in the reduction of the synthesis of lipoproteins. Reports also suggest that bile acids secretion is also found to be decreased by CCl₄ (Honma and Suda, 1997).

Being a vital component of biomembranes, the phospholipids are more prone to CCl₄-induced lipid peroxidation compared to other lipid types (Morrow et al., 1992). An increase in phospholipase activity may probably cause a concomitant decrease of phospholipids concentration in liver and kidney (Lamb et al., 1988). Extensive conversion of phospholipids into triglycerides is normal during lipoprotein metabolism (Wiggins and Gibbons, 1996). Triglyceride export associated with lipoprotein, as inhibited by CCl₄ becomes a reason for releasing of more phospholipids from these tissues.

Results of the current study disclosed that Azima tetracantha leaves extract significantly reduced serum levels of lipid biomarkers such as triglycerides, total cholesterol, and LDL, while it produced a noticeable elevation in serum level of HDL, which probably might be assigned to the role played by flavonoids through its antioxidant mechanism. By counterpoising the polyunsaturated fatty acid radicals, by obstructing the chain reactions and by suppressing the impact of non-essential fatty acids, flavonoids are found to be acting as inhibitors of lipid peroxidation which, probably has decreased the serum and tissue levels of cholesterol and triglycerides. Similar results were also shown by (Wang et al., 2011) who stated that hesperidin effectively reduced the steatosis of fatty liver, liver
weight, adipose tissue, and serum total cholesterol concentrations in rats, nourished with high cholesterol diet and reduced the probability of cardiovascular diseases.

CONCLUSIONS

Analyzing the results, it can be conjectured that Azima tetracantha leaves extract treatment shows significant improvement of lipid parameters in hyperlipidemic rats suggesting the therapeutic potential of using the same as a medicinal plant for managing hyperlipidemia and alleviating the risk of Cardiovascular Diseases (CVD).

Conflict of interest

The authors declare no conflicts of interest with relevance to the authorship and publication of this text.

REFERENCES


Table 1: The lipid profile in control and experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Free fatty acids (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>156.67±1.50a</td>
<td>91.6±1.1a</td>
<td>78.5±0.76a</td>
<td>90.8±0.7a</td>
<td>99.00±0.8641.97±1.08</td>
<td>15.70±0.15</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>410.83±1.54b</td>
<td>114.6±1.3a</td>
<td>370.5±1.48a</td>
<td>141.0±1.1b</td>
<td>48.67±0.88288.07±1.76</td>
<td>74.10±0.30</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>378.33±1.17c</td>
<td>107.3±0.8a</td>
<td>328.17±0.83b</td>
<td>109.5±0.8c</td>
<td>60.33±0.67252.37±1.79</td>
<td>65.63±0.17</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>300.00±1.39c</td>
<td>101.5±1.4c</td>
<td>279.50±1.23c</td>
<td>98.1±0.1d</td>
<td>77.33±0.88166.77±1.78</td>
<td>55.90±0.25</td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>224.00±1.06d</td>
<td>95.0±0.8a</td>
<td>160.33±1.23d</td>
<td>94.2±0.6e</td>
<td>88.33±0.76103.60±1.47</td>
<td>32.07±0.25</td>
<td></td>
</tr>
<tr>
<td>Group VI</td>
<td>197.67±1.84e</td>
<td>92.9±0.6a</td>
<td>137.17±0.95</td>
<td>89.5±0.6f</td>
<td>94.17±0.7976.07±2.37</td>
<td>27.43±0.19</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD for six rats. Mean values within the row followed by different letters (Superscript) are significant (P < 0.05) level difference from each other and same letters are non-significant by Duncan’s multiple range test (DMRT).


Ridgway, N. D. 2013. The role of phosphatidylcholine and choline metabolites to cell proliferation and survival, volume 48.


