5. Antihyperlipidemic activity

5.1 Introduction

Lipids are very diverse in both their respective structures and functions. These are insoluble in water. They are however soluble in other organic solvents such as ether, acetone and other. Major lipid groups include fats, phospholipids, steroids and waxes. The main biological functions of lipids include energy storage, signaling, and acting as structural components of cell membranes. In living cells, processes of carbohydrate metabolism, lipid metabolism and energy metabolism are closely related. Metabolic syndrome (MS), such as diabetes, obesity, hyperlipidemia and hypertension is more or less, associated with abnormal lipid metabolism. The accumulation of nutrients such as lipids and caloric surplus leads to abnormal lipid and ectopic fat accumulation, which is a fundamental component of metabolic disease. Elevated serum total cholesterol (TC), low density lipoproteins (LDL), very low density lipoprotein (VLDL) and decrease high density lipoprotein (HDL) are the major risk factors for coronary heart diseases and chronic degenerative disease such as atherosclerosis (Bertges, 2010, Rerkasan et al. 2008, Kaesancini, 1994). Recent findings indicated that some of medicinal herbs or drugs, in addition to their lipid lowering ability, can also reduce the production of reactive oxygen species and increase the resistance of plasma lipoprotein to oxidation that may contribute to their effectiveness at preventing atherosclerotic disease (Kim et al. 2003, Rosenson, 2004).

A systematic preclinical testing of extracts under investigation is very much essential to prove the safety and efficacy in the management of the disease for which it is
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developed for. The present study was, therefore, undertaken to study the *M. azedarach* Linn. for its lipid lowering activity in hyperlipidemic rats.

5.1.1 **Evaluation of *M. azedarach* L. for lipid lowering activity**

For the study of anti hyperlipidemia, animal model that satisfied the following conditions was related.

- The animals should develop hyperlipidemia rapidly and show reproducibility
- Pathological changes in the liver/body should result from hyperlipidemia
- The symptoms should be ameliorated / prevented by drug treatment and should be effective in human beings
- Drug dosages should approximate the optimum therapeutic range for human, based on the test animal weight

5.2 **Experimental methods**

5.2.1 **Selection of laboratory model**

Animal such as rabbits, rats and syrian hamsters have been used in experimental study of hyperlipidemia. The hepatic system of rats resembles human hepatic system in characteristics, thus, rats were employed in this present study.

We selected two animal models for screening.

1. Triton WR-1339 induced hyperlipidemia (acute model)
2. High fat diet induced hyperlidemia (chronic model)

5.2.2 **Animals**

Male Wistar albino rats obtained from Central Animal Research Facility (CARF) of manipal university, were acclimatized to the experimental room having temperature 25 ± 2°C, controlled humidity conditions and 12 h light-dark cycle. The rats were fed with commercially available rat standard pelleted diet and water *ad libitum*. Study was conducted after obtaining ethical committee clearance from the
5.2.3 Acute toxicity study

Acute toxicity study was conducted in Wistar Albino rats as per OECD guidelines 423. The test extracts were administered orally to overnight fasted animals at the dose of 2000 mg/kg b.w. Animals were observed continuously for initial period of 4 h, intermittently for the next 6 h, later at 24 h and 48 h, followed up to 14 days following the drug administration. The parameters observed were

- **Behavioural profile**
  
  Awareness: Alertness, visual placing, stereotypy, passivity
  
  Mood: Grooming, restlessness, irritability, fearfulness

- **Neurological profile**
  
  Motor activity: spontaneous activities, reactivity, touch, response, pain response, startle response, tremor, gait, grip strength, pinna reflex, and corneal reflex

- **Autonomic profile**
  
  Writhing, defecation, urination, pile erection, heart rate, respiratory rate

The dose level of extracts used for the pharmacological study was 1/10th and 1/20th of the maximum tolerated safe dose found from acute toxicity studies. These were administered once daily by oral route.
5.2.4 Experimental models

5.2.4.1. Triton WR-1339 induced hyperlipidemia (Acute model)

This model is widely used in order to screen natural or synthetic drugs. Triton WR-1339, a non-ionic detergent (oxy ethylated tertiary octyl phenol formaldehyde polymer) can causes acute hyperlipidemia. It blocks the clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals (Schurr, 1950, James Eliza, 2009, Abe, 2007).

Triton WR-1339 has a direct inhibitor effect on the lipoprotein lipase in muscle and adipose tissue. This property of Triton WR-1339 is the basis for its extensive usage as a model of acute hyperlipidemia.

5.2.4.1.1 Experimental protocol

**Triton WR -1339 induced hyperlipidemia**

In this model, animals were randomly divided into 13 groups of six animals each. Group 1 served as normal control group whereas other groups (2-13) were induced hyperlipidemia by administration of intraperitoneal (i.p) injection (200mg/kg) of Triton WR 1339 on 7th day. Group 2 was served as induction control, whereas group 13 served as standard control and received atorvastatin (0.4mg/kg). The other groups (3 to 12) were considered as treatment groups, treated with two doses of five different extracts PEMA, CHLMA, EAMA, MEMA and AQMA of *M. azedarach*. Group 1 and 2 received only vehicle during the prophylactic treatment period. Group 3 to 13 were given prophylactic dose for 7 consecutive days.

Group 1: Normal control rats, received normal saline only p.o

Group 2: Hyperlipidemic control received only vehicle p.o

Group 3: MA Pet. ether extract of 100 mg/kg p.o

Group 4: MA pet. ether extract of 200 mg/kg p.o
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Group 5: MA chloroform extract of 100 mg/kg p.o
Group 6: MA chloroform extract of 200 mg/kg p.o
Group 7: MA ethyl acetate extract of 100 mg/kg p.o
Group 8: MA ethyl acetate extract of 200 mg/kg p.o
Group 9: MA alcoholic extract of 100 mg/kg p.o
Group 10: MA alcoholic extract of 200 mg/kg p.o
Group 11: MA aqueous extract of 100 mg/kg p.o
Group 12: MA aqueous extract of 200 mg/kg p.o
Group 13: Atorvastatin 0.4 mg/kg p.o

On 8th day or after 24 h of induction, blood samples were collected from all animals by retro orbital puncture under slight anaesthesia and serum were separated and used for biochemical estimations such as high density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC) were estimated by using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany and protocol from manufacturer) using auto analyser (Cobas c111, Roche) in FIST-DST Lab, MCOPS, Manipal. very low density lipoproteins (VLDL), low density lipoproteins (LDL), TC/HDL, LDL/HDL were calculated using the Friedewald formula. The results were statistically analysed by one way ANOVA followed by Tukeys post hoc using Graph pad prism 5 statistical software.
5.2.4.2 High fat diet (HFD) induced hyperlipidemia (Chronic Model)

Hypercholesterolemia in rodents is induced by supplementing cholesterol or saturated fats into laboratory rodent chow (Vogel et al., 2008). Excessive cholesterol feeding leads to susceptibility to hypercholesterolemia and arteriosclerosis. High dietary fat intake was found to promote the development of obesity and hyperlipidemia in both humans and rodents by altering the plasma cholesterol and triglyceride levels (Bray et al. 2004, Golay and Bobbioni, 1997), which could then lead to a higher risk for various metabolic syndromes, including cardiovascular diseases, fatty liver disease, dyslipidemia and type 2 diabetes mellitus (Formiguera and Canton, 2004, Sanchez et. al., 2011, Stapleton et al. 2008).

Therefore, a therapeutic approach for improving lipid metabolism and preventing hyperlipidemia are of great importance in order to control the rising prevalence of CVD. Drugs that have antihyperlipidemic action are much needed. Therefore, this approach has been chosen. (Ban et.al., 2012)

The experimental model selected for the present study was high fat diet induced hyperlipidemia in rats. This animal model mimics human hyperlipidemia, inducing radicals showing the signs of oxidative stress (Sai Krishna et.al, 2010). The present study was aimed to evaluate the serum lipid parameters in the high fat diet (HFD) induced hypercholesterolemic rats after administrating the M. azedarach (M.A.) leaf extracts after 4 weeks.

5.2.4.2.1 Preparation of High Fat Diet (HFD)

Exactly weighed 610 g of powdered NPD, 5 g deoxycholic acid and 5 g cholesterol were added and mixed. After complete mixing the above, 90 g fructose was added and mixed well, later 280 g of coconut oil was added slowly into the above powder
with constant mixing, after complete mixing to get dough mass, made in to uniform size balls, stored in refrigerator (Dabhi, 2008, Xu and Liu, 2009).

<table>
<thead>
<tr>
<th>Table 5.1: Composition of Normal Pellet Diet</th>
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</thead>
<tbody>
<tr>
<td>Nutrients</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Crude Fat</td>
</tr>
<tr>
<td>Crude fibre</td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Phosphorus</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5.2: Composition of High Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Powdered Normal pellet diet</td>
</tr>
<tr>
<td>Oil</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>D(-) Fructose</td>
</tr>
</tbody>
</table>

5.2.4.2.2 Experimental protocol

In-house laboratory bred healthy male rats were used for the experiment because, besides preventing environmental variation, inbreeding provides a homogenous population of animals for experiments. Animals were obtained from Central Animal Research Facility (CARF) of Manipal University, Manipal and were acclimatized to the experimental room having temperature 25±2°C, controlled humidity conditions and 12 h light-dark cycle. The rats were fed with commercially available rat standard pelleted diet and water *ad libitum.*

Animals were grouped based on their body weight before feeding the HFD. Group 1 animals are considered as normal control and were fed with (NPD) normal pellet diet. The other animals were fed with HFD for 4 weeks. Total cholesterol (TC) level in HFD fed animals were estimated and regrouped at the end of 4\textsuperscript{th} week based on TC levels. Group 2 served as induction control and received vehicle only. Group 3 received atorvastatin (0.4mg/kg), group 4-7 were treated with aqueous and methanolic extracts of *M. azedarach* at two dose level for next 30 days (5\textsuperscript{th} week to 8\textsuperscript{th} week).
The normal control group and hyperlipidemic control groups received only vehicle. The animals received respective treatments for 4 weeks.

Group 1: Normal rats, received normal chow and served as normal control

The HFD-fed rats were randomised into following groups

Group 2: HFD fed received normal saline p.o
Group 3: MA aqueous extract 100 mg/kg p.o
Group 4: MA aqueous extract 200 mg/kg p.o
Group 5: MA alcoholic extract 100 mg/kg p.o
Group 6: MA alcoholic extract 200 mg/kg p.o
Group 7: Atorvastatin 0.4 mg/kg p.o

At the end of the treatment schedule on 30th day, animals were fasted overnight and blood samples were collected from all animals by retro orbital puncture under slight anaesthesia and serum were separated by centrifugation and subjected for fasting lipid profiles analysis. Animals were sacrificed and organs like aorta, heart, liver, pancreas, spleen, kidney and abdominal fat pad of each animal was carefully isolated, weighed and used for histopathological studies. The relative organ to body weight was calculated.

5.2.4.3 Biochemical analysis

From the collected blood serum, the biochemical markers such as high density lipoprotein (HDL), triglycerides (TG), total cholesterol (TC) were estimated by using commercial kits (Roche Diagnostics GmbH, Mannheim, Germany and protocol from manufacturer) using auto analyser (Cobas c111, Roche) in FIST-DST Lab, MCOPS, Manipal. Very low density lipoproteins (VLDL), low density lipoproteins (LDL), TC/HDL, LDL/HDL were calculated using the Friedewald formula.
5.3 Statistical analysis

All the datas were expressed as mean ± SEM

The results were statistically analysed by one way ANOVA followed by Tuckey`s post hoc using Graph pad prism 5 statistical software. San Diego California USA, www.graphpad.com. $p<0.05$ was considered as statistically significant.
5.4 Results

5.4.1 Acute toxicity study

The acute toxicity test was executed as per OECD guidelines adoption 423 in overnight fasted Wistar albino rats at 2000 mg/kg body weight. Oral administration of different extracts showed neither any sign of clinical abnormality nor any mortality. Hence the sealing doses were considered safe for each extract. One tenth and 1/20\textsuperscript{th} of the safe dose was selected for lipid lowering activity.

5.4.2 Triton WR-1339 induced hyperlipedemia

Administration of tritonWR-1339 to normal rats in the dose of 200 mg/kg caused significant increase in lipid profile such as TG, TC (p<0.001) as compared to normal control rats. Pre treatment of aqueous and methanolic extracts in the dose of 100mg/kg b.w and 200 mg/kg b.w p.o significantly modified the increased level of TG, TC (p<0.001). Treatment with standard drug (Atorvastain 0.4 mg/Kg) significantly decreased the level of TG, TC (p<0.001). Pre-treatment of aqueous (AQ) 100 and 200 mg/kg b.w methanolic extract (MeOH) 100 and 200 mg/kg b.w decreased the level of TG, TC, (p<0.001) which was increased due to triton administration, whereas pre-treatment with petroleum ether(PEMA), chloroform(CHLMA), ethyl acetate(EAMA) extracts did not modified the triton induced hyperlipidemia. However pre-treatment with \textit{M. azedarach} extracts have shown significant increase in HDL compared to triton induced hyperlipidaemic group (Table5.3).

5.4.3 High fat diet induced hyperlipedemia

Chronic administration of HFD to normal animals causes significant rise (p<0.001) in the level of TG, TC as compared to normal control rats (Table no .), whereas lower levels of HDL compared to NPD-fed rats. TC / HDL and LDL / HDL ratios
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were significantly elevated. Simultaneous administration of aqueous and methanolic extracts in the dose of 100mg/kg b.w and 200 mg/kg b.w p.o showed significantly decreased the TG,TC (p<0.001) as compared to HFD treated rats(groups). Treatment with reference drug (Atorvastain 0.4 mg/Kg) also decreased the level of TG, TC (p<0.001) as compared to HFD treatment group. Moreover Atorvastain also significantly increased HDL levels (Table 5.4).

Animals fed with HFD showed significant increase in body weight as compared to normal (NPD) treated groups. Chronic administration of aqueous and alcoholic extracts of MA at the dose of 100 and 200 mg/kg significantly reduced the increased body weight due to HFD administration compared to HFD fed control group.

5.2.4 Histopathological observations

(A) In normal control group showing normal architecture; with no inflammation, no fibrosis, no fatty changes and necrosis

(B) hyperlipidemic group showing; altered architecture. Hepatocytes focally show fatty vacuoles, focal lymphocytic infiltration around bile ductules. Nucleus is pushed by fatty vacuoles to one side with no fibrosis, inflammation or necrosis. This feature favor fatty changes in the liver with fatty infiltration and granular degeneration (C)

There was no alterations found in the liver histology for the group treated with standard drug atorvastatin showing negligible cytoplasmic fatty infiltration and granular degeneration; (D) Group treated with *M. azedarach* aqueous extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration

(E) Group treated with *M. azedarach* methanolic extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration
**Table 5.3:** Effect of different extracts of *M. azedarach* on serum lipid parameters in Triton WR-139 induced hyperlipidemia in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>VLDL</th>
<th>LDL</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>56.9 ± 2.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.27 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.23 ± 1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.38 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.66 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.22 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triton</td>
<td>233.8 ± 2.65</td>
<td>710.0 ± 64.69</td>
<td>15.28 ± 1.94</td>
<td>46.76 ± 0.21</td>
<td>648.06 ± 7.81</td>
<td>46.47 ± 0.14</td>
<td>42.41 ± 0.14</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>67.27 ± 4.053&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.4 ± 6.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.83 ± 14.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.45 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.116 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48 ± 0.16</td>
<td>1.09 ± 0.02</td>
</tr>
<tr>
<td>PE 100</td>
<td>174.8 ± 16.32</td>
<td>481.9 ± 41.38</td>
<td>26.3 ± 6.129</td>
<td>43.86 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.13 ± 5.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.63 ± 0.36</td>
<td>3.84 ± 0.32</td>
</tr>
<tr>
<td>PE 200</td>
<td>182.3 ± 8.66</td>
<td>471.3 ± 26.11</td>
<td>24.51 ± 9.29</td>
<td>49.44 ± 1.26</td>
<td>119.34 ± 0.94</td>
<td>6.96 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CHL 100</td>
<td>197.6 ± 13.26</td>
<td>608.6 ± 87.59</td>
<td>28.32 ± 2.61</td>
<td>40.72 ± 2.05</td>
<td>78.73 ± 0.81</td>
<td>5.42 ± 0.01</td>
<td>2.91 ± 0.27</td>
</tr>
<tr>
<td>CHL 200</td>
<td>160.7 ± 11.37</td>
<td>636.9 ± 85.4</td>
<td>27.05 ± 3.74</td>
<td>46.66 ± 0.24</td>
<td>116.12 ± 0.32</td>
<td>6.75 ± 2.01</td>
<td>4.10 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EA 100</td>
<td>177.3 ± 8.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>574.9 ± 85.94</td>
<td>28.15 ± 13.51</td>
<td>43.2 ± 0.62</td>
<td>68.18 ± 0.73</td>
<td>4.57 ± 2.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.18 ± 5.06</td>
</tr>
<tr>
<td>EA 200</td>
<td>166.01 ± 5.62</td>
<td>474.4 ± 88.81</td>
<td>31.22 ± 2.41</td>
<td>34.9 ± 0.34</td>
<td>82.97 ± 1.49</td>
<td>5.05 ± 0.14</td>
<td>2.85 ± 0.02</td>
</tr>
<tr>
<td>MeOH 100</td>
<td>141.6 ± 15.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>309.8 ± 24.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.13 ± 1.55</td>
<td>33.36 ± 0.32</td>
<td>78.61 ± 0.21</td>
<td>4.45 ± 1.06</td>
<td>2.42 ± 0.61</td>
</tr>
<tr>
<td>MeOH 200</td>
<td>136.9 ± 11.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>241.1 ± 36.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.43 ± 3.06</td>
<td>37.12 ± 3.01</td>
<td>62.8 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.81 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQ 100</td>
<td>145.2 ± 13.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>298.2 ± 47.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.68 ± 5.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.94 ± 4.02</td>
<td>75.06 ± 0.46</td>
<td>4.82 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.61 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQ 200</td>
<td>115.2 ± 8.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>173.6 ± 35.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.8 ± 2.468</td>
<td>43.86 ± 1.09</td>
<td>94.13 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.63 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.84 ± 1.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are means ± SEM. <sup>c</sup>p<0.05, <sup>b</sup>p<0.01, <sup>a</sup>p<0.001 When compared with disease control group. One way ANOVA followed by Tukeys post test.
### Table 5.3 Effect of different extracts of *M. azedarach* on serum lipid parameters in on HFD fed hyperlipidaemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
<th>TC/HDL</th>
<th>LDL/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52.74 ± 1.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.73 ± 3.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.01 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.783 ± 2.071&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.95 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.39 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.081 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD</td>
<td>91.16 ± 4.50</td>
<td>135.9 ± 10.23</td>
<td>23.02 ± 1.17</td>
<td>12.43 ± 5.73</td>
<td>27.18 ± 2.04</td>
<td>1.90 ± 2.07</td>
<td>0.33 ± 0.45</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>67.06 ± 2.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.65 ± 3.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.68 ± 2.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.067 ± 1.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.13 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.63 ± 0.073&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQ100</td>
<td>72.78 ± 2.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.9 ± 4.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.53 ± 3.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0 ± 2.53</td>
<td>15.38 ± 0.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.79 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 1.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQ200</td>
<td>68.32 ± 3.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.05 ± 5.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.33 ± 1.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.227 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.81 ± 1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.61 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53 ± 2.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ME100</td>
<td>84.59 ± 4.05</td>
<td>91.62 ± 4.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.21±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.055 ± 3.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.32±0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ME200</td>
<td>76.22 ± 2.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.1 ± 9.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.28 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.29 ± 6.5</td>
<td>18.62±1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are means ± SEM. <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05. When compared with disease control group. One way ANOVA followed by Tukeys post test.
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Fig 5.2: Absolute weights of the different organs in HFD
Fig 5.1: Histomicrographs showing histopathological changes in rat Liver
(Histomicrographs showing histopathological observations in the Liver of the animals treated with *M. azedar*ch extracts, standard drug and the normal group)
(A) control group showing normal architecture; with no inflammation, no fibrosis, no fatty changes and necrosis
(B) hyperlipidemic group showing; altered architecture. Hepatocytes focally show fatty vacuoles, focal lymphocytic infiltration around bile ductules. Nucleus is pushed by fatty vacuoles to one side with no fibrosis, inflammation or necrosis. This feature favor fatty changes in the liver with fatty infiltration and granular degeneration (C)
There was no alterations found in the liver histology for the group treated with standard drug atorvastatin showing negligible cytoplasmic fatty infiltration and granular degeneration; (D) Group treated with *M. azedarach* aqueous extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration (E) Group treated with *M. azedarach* methanolic extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration

**In conclusion**, the present study focused on the estimation of phytochemicals, *in vitro* antioxidant study and lipid lowering activities of different extracts of *M. azedarach*. Ethyl acetate and methanolic extracts showed significant antioxidant activity. The present study offers data for supporting the use of *M. azedarach* extracts as natural antioxidant agents, nevertheless, the different extracts also showed the presence of bioactive compounds like β-sitisterol, lupeol, rutin and quercetin and that this plant represent an important source of flavonoid, phenolic compounds. This results suggest that *M. azedarach* has the potential to be a candidate as a lipid lowering agent. Experimental results suggest that *M. azedarach* has the potential to be a candidate as a lipid lowering agent. Mechanism of action needs to be envisaged.
Antihyperlipidemic activity

References


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Antihyperlipidemic activity


