Hypolipidemic Efficacy of *Artemisia absinthium* Extracts in Rabbits

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Abstract: The hypolipidemic effects of 70% ethanol extract of *Artemisia absinthium* in hypercholesterolemic -fed rabbits. Hypercholesterolemia was induced in male rabbits by high cholesterol diet (HCD) (350 mg/kg) for 8 weeks. Hypercholesterolemic rabbits were allocated into groups, treated with simvastatin (SIM 5 mg/kg), different extracts of *Artemisia absinthium* at two doses of 500, 1000 mg/kg. A normal control group and an HCD control one were used for comparison. During the hall period of the experiment blood samples were collected and serum was analyzed for lipid profile. At the end of the experiment the animals were sacrificed; the heart and the liver were collected and stored at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were performed for total cholesterol, total triglycerides, Phospholipid, LDL-C, HDL-C, VLDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatine kinase (CK) and total protein. The extract induces a significant decrease in serum cholesterol, triglycerides and CK levels. Blood levels of AST, ALT, triglycerides and total protein unchanged. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. The results concluded that *Artemisia absinthium* ethanolic extract (500, 1000 mg/kg) have potent antihyperlipidemic activity in high cholesterol diet induced hyperlipidemia model and which is equipotent activity when compared with control group.

Key words: *Artemisia absinthium* • Anti-Hyperlipidemic Activity • Triglyceride • Cholesterol • High Cholesterol Diet Induced Hyperlipidemia

INTRODUCTION

Excessive quantities or improper types of lipid-intake may result in hyperlipidemia which is characterized by an abnormal elevation in one or more of the serum lipids such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). Hyperlipidemia is considered to be a major risk factor for cardiovascular diseases including atherosclerosis, myocardial infarction, heart attacks and cerebrovascular diseases [1]. Today in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths. Hypercholesterolemia and hypertriglycerideremia are independent risk factors that alone or together can accelerate the development of atherosclerosis and progression of atherosclerotic lesions [2]. High cholesterol diet increase serum total cholesterol (TC) and LDL-C levels [3]. Oxidative modification of LDL-C plays a major role in the pathogenesis of atherosclerosis. The first stage of atherogenesis is characterized by an influx and accumulation of LDL-C in the intima, followed by recruitment of blood-derived monocytes and lymphocytes to the developing lesion [4]. Subsequently, LDL-C is oxidized by free radicals; ox-LDL-C induces a multitude of cellular responses which lead to vascular dysfunction [5].

*Artemisia absinthium* L. (Family: Asteraceae) known as wormwood is an aromatic, perennial small shrub. Ethnopharmacological literature documents the use of *Artemisia absinthium* in Europe and Pakistan as an antiseptic, antispasmodic, febrifuge, cardiac stimulant, for the restoration of declining mental function and inflammation of the liver and to improve memory [6-8].

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Traditional Chinese medicine practitioners use the plant for treating acute bacillary dysentery. Cancers and neurodegenerative diseases [9-10].

*Artemisia absinthium* has been reported to enhance the cognitive ability as evidenced by its nicotinic and muscarinic receptor activity in homogenates of human cerebral cortical membranes [7]. Free radical scavenging activity of *Artemisia absinthium* extracts have been reported both *in vitro* and *in vivo* [11, 12]. Moreover, it has been reported that ethanol extract of this plant enhanced neuritis outgrowth induced by nerve growth factor and PC12D cells [13]. Although the aforementioned literature survey reveals that *Artemisia absinthium* possesses antioxidant, anti-inflammatory and cognition enhancement activity Neuroprotective [14], Genotoxicity activity of *Artemisia absinthium* extracts have been reported [15].

The plant has undergone extensive phytochemical investigations and the presence of a variety of chemical constituents such as ascorbic acid [16], flavonoids [17], carotenoids [18], tannins [16] and lignans [19] have been identified.

Therefore, the current study was designed to evaluate the hypolipidimic effect of *Artemisia absinthium* in hyper-cholesterolemic rabbits in order to verify the activities for which the plant is used in traditional medicine. Also, the toxicity of different extracts was evaluated.

**MATERIALS AND METHODS**

**Animals:** Prior to the initiation of the experiment an ethical clearance for performing the experiments on animals was obtained from Institutional Animal Care and Use Committee (IACUC). Adult healthy New Zealand White rabbits (2-2.5 kg) were housed individually in metallic cages left for 7 days prior to the study to acclimatize and received standard pellets (15% protein, 2.5% lipid, 15% cellulose, 14% clay, 13% water) [20]. The animals were maintained on normal light-dark schedule and temperature 25 ± 3°C throughout the experiment and given free access to water. All experimental protocols were approved by the Institutional Animal Care. This diet was supplemented with green leafy vegetables and water *ad libitum*. The average consumption of diet was calculated 200 g day.

**Experimental Design:** Rabbits were fed either a standard chow diet (control group, n = 8) or a high-cholesterol diet (HCD, n = 32) consisting of standard chow supplemented with cholesterol (350 mg/kg/day) (Oxford Lab, Mumbai, India), dissolved in cotton seed oil for 8 weeks. This dose was proved to induce hypercholesterolemia [21]. Cotton seed oil is a natural oil that contains a high proportion of polyunsaturated fatty acids and proved to have no effects on the tissues [22]. Hypercholesterolemic rabbits were, then, randomly divided into nine treatment groups. During treatment, all animals continued to receive the HCD. Animals were treated once daily by oral gavage with the compounds suspended in Tween 20 (Vehicle) according to the following schedule:

- **Group 1 (Control group):** Fed a standard pellet 100 g/kg/d.
- **Group 2 (HCD):** Fed a HCD and received no additional treatment.
- **Group 3 (SIM):** HCD received simvastatin at doses of 5 mg/kg [23].
- **Group 4 (A. absinthium 500):** HCD received 500 mg/kg *Artemisia absinthium* extract.
- **Group 5 (A. absinthium 1000):** HCD received 1000 mg/kg *Artemisia absinthium* extract.

**Toxicity Study:** The toxicity of four used *Artemisia absinthium* extracts were tested using four doses (100, 250, 500 and 1000 mg/kg) (Three rabbits for each dose). Three control rabbits were kept under the same conditions without any treatments. The animals were observed continuously during the first hour and then every hour for 6 hrs, then after 12 and 24 hrs and finally after every 24 hrs, up to 3 weeks, for any physical signs of toxicity such as writhing, gasping, salivation, diarrhea, cyanosis, pupil size, any nervous manifestations, or mortality [24].

**Plant and Treatment:** *A. absinthium* plants were recognized and collected from the Irbid area located to the north of Jordan. The plant was chopped then grinded using electrical grinder until powder was obtained. Powder was extracted by water-ethanol mixture (70:30 V/V) for 6 hrs. This step was repeated three times then the filtrate was pooled and concentrated under vacuum keeping a temperature less than 50°C. The concentrate was dissolved in a normal saline and used. The extract, 500 and 1000 mg/kg, dissolved in 1 ml normal saline was administered orally to rats using animal feeding Intubation’s needles (Popper and Sons, New York).
Table 1: Effect of Artemisia absinthium (70% EtOH) extract on body, liver and heart weight in hypercholesterolemic rabbits. (8 animals per treatment)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (kg)</th>
<th>% Body weight</th>
<th>Liver</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.13±0.28</td>
<td>2.21±0.18</td>
<td>2.75±0.25</td>
<td>0.27±0.23</td>
</tr>
<tr>
<td>HCD</td>
<td>2.01±0.14</td>
<td>2.14±0.71</td>
<td>3.30±0.78</td>
<td>0.30±0.68</td>
</tr>
<tr>
<td>HCD + SIM 5 mg/kg</td>
<td>2.16±0.46</td>
<td>2.05±0.32</td>
<td>2.71±0.81</td>
<td>0.25±0.18</td>
</tr>
<tr>
<td>HCD + A. absinthium 500 mg/kg</td>
<td>2.10±0.19</td>
<td>1.89±0.57</td>
<td>2.80±0.69</td>
<td>0.28±0.18</td>
</tr>
<tr>
<td>HCD + A. absinthium 1000 mg/kg</td>
<td>2.03±0.66</td>
<td>1.93±0.44</td>
<td>2.69±0.81</td>
<td>0.24±0.45</td>
</tr>
</tbody>
</table>

Rabbits were treated for four weeks. Results were expressed as mean ± SEM and analysed using one-way ANOVA followed by Duncan's post-hoc test. *P < 0.05 compared to control group, †P < 0.05 compared to HCD group, ‡P < 0.01 compared to HCD group.

Blood Sampling and Biochemical Analysis: At the end of the study, rabbits were fasted overnight, anesthetized with thiopental sodium (50 mg/kg) [25]. Blood samples were collected by cardiac puncture. Blood was centrifuged at 2000 × g for 15 min after 30 min of collection and stored at -80°C until assayed. And the heart, the aorta and the liver were removed, cleaned from the fat and adhering connective tissue and stored at -20°C until assayed. Biochemical analysis of tissue (liver and heart muscle) were made for cholesterol [26], phospholipids [27] and triglyceride [28].

Determination Serum Lipid Profile and Atherosclerosis Index: Serum triglycerides (Tgs), Total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) were measured colorimetrically using assay kits from (Stanbio, Texas, USA) according to the manufacturer instructions using V-visible spectrophotometer (UV-1601PC, Shimadzu, Japan). LDL Cholesterol was calculated by using Friedewald’s formula [29].

- LDL (mg/dl) = TC-(HDL+VLDL), VLDL cholesterol was calculated as TG/5 and LDL.
- Atherosclerosis index was calculated by the formula [30]:
  Atherosclerosis index = (serum TC- HDL-C)/HDL-C.

Determination of Liver Enzymes: Commercial kit purchased from Bio Med Diagnostics (Oregon, USA), was used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) using UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan).

Measurement of Blood Urea Nitrogen (Bun), Creatinine and Total Protein: Creatinine and BUN were determined enzymatically using commercially available kits (Spinreact, Gerona, Spain). Total Protein was determined using (BioSystems S.A.Costa Brava30, Barcelona-Spain), according to the manufacturer’s protocol. Serum levels of BUN, creatinine and total protein were determined by colorimetric methods using UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan).

Statistical Analysis: All data were expressed as mean ± SEM and analyzed using the Statistical Package of Social Sciences (SPSS) program version 17, (Chicago, IL, USA). For all parameters, comparisons among groups were carried out using oneway analysis of variance (ANOVA) followed by Dunnat's multiple comparison tests [31]. All P values reported are two-tailed and P < 0.05 was considered significant.

RESULTS

Toxicity Study: The toxicity study revealed the non-toxic nature of extracts of Artemisia absinthium at doses up to 1000 mg/kg. Rabbits did not show any drug-induced physical signs of toxicity during the whole experimental period and no deaths were reported.

Effect of A. Absinthium Ethanol Extract on Body Weight: At the end of study period, hypercholesterolemic rabbits showed a non-significant reduction in the body weights was noticed in rabbits fed with cholesterol diet and later treated with SIM and Artemisia absinthium extract (Groups D and E) in comparison with the initial body weights. A non-significant change in heart weight of cholesterol fed rabbits. Liver weight was significantly increased in cholesterol fed rabbits (Table 1).

Effect of A. Absinthium Ethanol Extract on Tissue Biochemistry: Tissue lipid was significantly increased by feeding HCD. Table 2 showed significant increase in total cholesterol, triglycerides and phospholipids. SIM AND Artemisia absinthium (70% EtOH) extract feeding (Groups D and E) resulted in a significant lowering of total cholesterol, triglycerides and phospholipids of liver and ventricular heart muscles in comparison with cholesterol fed rabbits. In group D the reduction was on higher side (Table 2).
Table 2: Effect of Artemisia absinthium (70% EtOH) extract on tissue lipids profile in hypercholesterolemic rabbits. (8 animals per treatment)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mg/g)</th>
<th>Triglycerides (mg/g)</th>
<th>Phospholipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver (g)</td>
<td>Heart (g)</td>
<td>Liver (g)</td>
</tr>
<tr>
<td>Control</td>
<td>9.1± 0.18</td>
<td>6.59± 0.66</td>
<td>3.71± 0.61</td>
</tr>
<tr>
<td>HCD</td>
<td>16.55± 0.7*</td>
<td>11.14 ± 0.33*</td>
<td>5.36 ±0.84*</td>
</tr>
<tr>
<td>HCD + SIM 5 mg/kg</td>
<td>9.53± 0.27†</td>
<td>7.18± 0.62 †</td>
<td>4.07± 0.93 †</td>
</tr>
<tr>
<td>HCD + A. absinthium 500 mg/kg</td>
<td>9.67± 0.13†</td>
<td>8.98±0.19†</td>
<td>4.46±0.42†</td>
</tr>
<tr>
<td>HCD + A. absinthium 1000 mg/kg</td>
<td>9.40± 0.12‡</td>
<td>6.94±0.7 ‡</td>
<td>3.92±0.74‡</td>
</tr>
</tbody>
</table>

Rabbits were treated for four weeks. Results were expressed as mean ± SEM and analysed using one-way ANOVA followed by Duncan's post-hoc test. *P < 0.05 compared to control group, †P < 0.05 compared to HCD group, ‡P < 0.01 compared to HCD group.

Table 3: Effect of Artemisia absinthium (70% EtOH) extract on serum lipid profile and atherosclerotic index in hypercholesterolemic rabbits (8 animals per treatment)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>LDL-cholesterol (mg/dl)</th>
<th>Atherosclerotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.2±1.85</td>
<td>77.15±5.33</td>
<td>182.32±4.3</td>
<td>37.85±2.11</td>
<td>16.34±1.87</td>
<td>32.52±8.35</td>
</tr>
<tr>
<td>HCD</td>
<td>611.35 ±2.23</td>
<td>205.67±4.12*</td>
<td>278.56±6.92*</td>
<td>27.5±2.93</td>
<td>42.35±1.23*</td>
<td>447.31±7.48*</td>
</tr>
<tr>
<td>HCD + SIM 5 mg/kg</td>
<td>345.37±7.81†</td>
<td>134.35±8.10†</td>
<td>186.31±6.92†</td>
<td>34.5±2.93†</td>
<td>28.81±3.35†</td>
<td>177.10±5.51†</td>
</tr>
<tr>
<td>HCD + A. absinthium 500 mg/kg</td>
<td>207.6±7.8†</td>
<td>163.41±8.11†</td>
<td>193.68±4.77†</td>
<td>31.53±2.18†</td>
<td>31.34±2.63†</td>
<td>136.22±5.51†</td>
</tr>
<tr>
<td>HCD + A. absinthium 1000 mg/kg</td>
<td>105.2 ±8.84‡</td>
<td>123.51±6.68‡</td>
<td>181.12±7.48‡</td>
<td>36.12±1.52‡</td>
<td>27.56±1.54‡</td>
<td>67.87±5.65‡</td>
</tr>
</tbody>
</table>

Rabbits were treated for four weeks. Results were expressed as mean ± SEM and analysed using one-way ANOVA followed by Duncan's post-hoc test. *P < 0.05 compared to control group, †P < 0.05 compared to HCD group, ‡P < 0.01 compared to HCD group.

Effect of Artemisia Absinthium Extracts on Serum Lipid Profile and Atherosclerosis Index: In the present study, the results obtained from exclusive high cholesterol fed rabbits were compared with those of the normal group. While the values for the group that were fed HCD plus extract of Artemisia absinthium leaves were compared with those of the normal group. (Table No. 3)

A seventh-fold increase was observed in serum cholesterol in treated rabbits fed with atherogenic diet. Table 3 shows a significant increase in TC, TG and LDL-C and a non-significant decrease in HDL-C in HCD group as compared to control group. SIM significantly ameliorated dyslipidemia, while serum HDL-C slightly (but not significantly) increased in rabbits treated with SIM. On the other hand, Artemisia absinthium reduce the serum level of TC, TG and LDL-C as compared to HCD group. Additionally, the difference in the calculated atherosclerosis index between the HCD group and the treated groups was significant (P< 0.05).

Effect of Artemisia Absinthium Extracts on Liver, BUN, Serum Creatinine and Total Protein: Feeding of the HCD for 8 weeks resulted in a non-significant increase in serum levels of ALT, AST and ALP compared with the normal control group. SIM and Artemisia absinthium extracts showed non-significant changes in these levels (Table 4). Hypercholesterolemic rabbits showed a non-significant change in BUN, serum creatinine and total protein as compared to normal controls. Oral administration of SIM and Artemisia absinthium extracts did not show any significant change in these serum levels (Table 4).

DISCUSSION

The purpose of this study was to evaluate the hypolipidemic effects of Artemisia absinthium in hypercholesterolemic-fed rabbits. Hyperlipidemia is a major risk factor that can facilitate the development of coronary artery diseases and progression of atherosclerotic lesions. As increase in lipid profiles is a contributing factor to the pathogenesis of atherosclerosis associated cardiac disorders [1]. The induction of hyperlipidemia, particularly hypercholesterolemia by feeding experimental animals a high cholesterol diet (HCD), has been suggested by many scientists as a reliable model for atherosclerosis in humans. HCD causes marked hypercholesterolemia i.e. increased level TC, LDL-C, VLDL-C. Elevated lipid level specially hypercholesterolemia results due to increased absorption in the gut or endogenous synthesis [32]. Therefore in this study we evaluated the hypolipidemic activity of Artemisia absinthium extract in rabbit’s model in which hyperlipidemia was induced by HCD. The increase in body weight after hyperlipidemia is generally known and it
Table 4: Effect of Artemisia absinthium (70% EtOH) extract on liver enzymes, BUN, serum creatinine and total protein in hypercholesterolemic rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum ALT (U/L)</th>
<th>Serum AST (U/L)</th>
<th>Serum ALP (U/L)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.43 ±1.3</td>
<td>56.78 ±2.66</td>
<td>65.50 ±2.66</td>
<td>32.26 ±6.3</td>
<td>1.46 ±1.9</td>
<td>8.72 ±1.9</td>
</tr>
<tr>
<td>HCD</td>
<td>38.15 ±2.8</td>
<td>65.83 ±6.87</td>
<td>70.52 ±6.87</td>
<td>33.68 ±7.66</td>
<td>1.41 ±4.3c</td>
<td>10.79 ±4.3</td>
</tr>
<tr>
<td>HCD + SIM 5 mg/kg</td>
<td>40.54 ±2.13</td>
<td>64.31 ±6.8</td>
<td>68.72 ±6.8</td>
<td>30.84 ±2.66</td>
<td>1.31 ±2.66</td>
<td>8.82 ±2.66</td>
</tr>
<tr>
<td>HCD + A. absinthium 500 mg/kg</td>
<td>33.24 ±2.8</td>
<td>62.45 ±4.11</td>
<td>66.92 ±4.11</td>
<td>34.17 ±5.22</td>
<td>1.32 ±1.44c</td>
<td>8.93 ±1.44</td>
</tr>
<tr>
<td>HCD + A. absinthium 1000 mg/kg</td>
<td>28.77 ±1.84</td>
<td>60.82 ±9.7</td>
<td>59.36 ±9.7</td>
<td>31.61 ±7.67</td>
<td>1.27 ±1.26</td>
<td>8.52 ±1.26</td>
</tr>
</tbody>
</table>

Rabbits were treated for four weeks. Results were expressed as mean ± SEM and analysed using one-way ANOVA followed by Duncan's post-hoc test. *P < 0.05 compared to control group, †P < 0.05 compared to HCD group, ‡P < 0.05 compared to HCD group.

can be used as one of the aspect of the animal models to
develop antiobesity agents similar to those of previous
study. A significant decrease in body weight was detected
in HCD control group compare to normal control. In
present study, however no favorable changes in body
weight were detected after A. absinthium leaves extract
dosing.

In the animal model used, the hypercholesterolemic
diet severely increased plasma TC and LDL-C levels
reaching about 8 times and 3 times the values in control
animals respectively, consistent with the results of
another study reported by Daradka et al. [33].
HCD induced also a moderate increase in TGs and a
non-significant decrease in HDL-C, consistent with the
results reported by Hernandez-Presa et al. [34] and
Nachtigal et al. [35]. As expected, SIM achieved a 53% and 78% decrease in TC and LDL-C levels respectively,
consistent with previous studies [36]. In accordance to
Tavridou et al. [37], non-significant changes were
observed following 4 weeks of treatment by SIM
regarding levels of HDL-C, whereas a significant but
modest decrease was observed in TG levels.

LDL levels in the hypercholesterolemic diet group
were higher than in the control group in spite of treatment
with SIM, probably due to the excessive amount of
cholesterol added to the diet. However, this gave us the
opportunity to examine the beneficial effects of SIM
additional to lowering of cholesterol levels. Results of
this study demonstrated that hypolipidaemic nature of A.
absinthium. The increased cholesterol levels were
brought to normal by adding of A. absinthium.

Serum cholesterol levels dropped significantly by
the end of the experiment. Similarly, phospholipids and
triglycerides levels were observed to be also reduced.
The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids.
A positive correlation between cholesterol plasma
concentration and the risk of coronary heart disease has been widely demonstrated by the lipid research Clinics
Primary Prevention Trials [38]. In order to find good
means to decrease plasma cholesterol level with minimal
toxicity. The level of cholesterol in lipoprotein fractions
has been shown to be a good indicator of atherosclerosis
risk in rabbits [39]. Significant lowering of cholesterol
after Artemisia absinthium feeding indicates a risk
reduction action. Plasma triglycerides and cholesterol
carry the highest risk for ischemic heart disease [40]. HDL
and LDL cholesterol are significant variables and indicator
for coronary heart disease [41]. It is reported that HDL is
inversely related to total body cholesterol.

Treatment with Artemisia absinthium extract reduces
serum cholesterol and triglyceride by 8 and 3.5 times,
respectively.

HDL alters the balance of unesterified cholesterol
between plasma and cell by increasing its utilization in the
lecithin cholesterol acyl transferase (LCAT) system to
form cholesterol ester which moves rapidly into the cells.
Decreased total cholesterol and phospholipid after
Artemisia absinthium extract feeding indicate the
anti-atherogenic or hypolipidaemic nature of the plant
product. Further reduction in total cholesterol, triglyceride
and phospholipids of liver and ventricular heart muscle
may be suggestive of a beneficial role of Artemisia
absinthium L. in hyperlipidaemic subject. The possible
mechanism of lipid alteration might be cholestatic effect
of Artemisia absinthium in liver enhanced removal or
catabolism of lipoproteins [42] and/or inhibition of
lysosomal lipid hydrolytic enzymes secreted by the liver
[43]. In conclusion Artemisia absinthium possesses
active hypolipidaemic constituents. Further chemical and
pharmacological investigations are in progress.

REFERENCES

Eclonia stolonifera and its constituents on Poloxamer 407-Induced Hyperlipidemic and


