Evaluation of Hypolipidemic Activity of Ionidium Suffruticosum (Ging.) in Rats Fed with High Fat Diet

A. Kottai Muthu* and D. Satheeshkumar

Abstract—The present study was designed to investigate the hypolipidemic effect of different extracts from whole plant of Ionidium suffruticosum (Ging.) in rats fed with high fat diet. There was a noticed increase in the body weight in HFD fed group (p<0.001), which was reduced by the administration of methanolic extract of Ionidium suffruticosum (250mg/kg). The elevated levels of total cholesterol, triglycerides, LDL-C and VLDL-C were observed in rats fed with high fat diet (group II). After the treatment of methanolic extract of Ionidium suffruticosum (250mg/kg/day) showed a significant (p<0.001) decrement in body weight, plasma total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C along with an increase in HDL-C when compared to HFD rats (group II). The similar result was not found in other two extract treatment groups. The methanolic extract of Ionidium suffruticosum could protect against atherosclerosis and decrease the atherogenic index than that of other extract treatment groups. Hypolipidemic activity of the methanolic extract of Ionidium suffruticosum in chronic hyperlipidemic rats validates it use traditionally as a part of folklore medicine in India, though there is no scientific evaluation to date.

Keywords— high fat diet, hypolipidemia, rats, Ionidium suffruticosum.

I. INTRODUCTION

Hyperlipidemia is a condition associated with increased levels of lipid and cholesterol in plasma leading to various physiological disorders including coronary artery disease [1]. Coronary artery disease cause more mortality and morbidity rate in both developed and developing nations [2]. Since synthetic drugs are shown more side effects, clinical importance of the herbal drugs has received considerable attention recent years [3] as medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesterolemic properties [4],[5].Nowadays there is an increasing interest toward the potential health benefits of medicinal plants. Ionidium suffruticosum (Ging.) it belongs to the family Violaceae known as Lakshmisheshta, Padmcharini or Purusharathna in Sanskrit, is an important plant in the Indian system of Medicine.

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It is widely used as traditional healers for the treatment of diseases like diabetes [6], male sterility [7], urinary tract infections and water retention [8]. The tender leaf stalks are used as demulcent; the roots are antigonorrhoeic, diuretic, bowel complaints and urinary problems [9]. It is also attributed to its anti-inflammatory, antitussive, antimicrobial and antiplasmodial action [10], [11]. It is used as anticonvulsant and free radical scavenging activity [12]. The aqueous and methanol leaf extracts possessed hypoglycemic activities [13]. Based on this information present study was designed to investigate the hypolipidemic activity of whole plant of Ionidium suffruticosum (Ging.) in rat fed with high fat diet.

II. MATERIAL AND METHODS

A. Plant materials

The whole plant of Ionidium suffruticosum (Ging) collected at Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The whole plant of Ionidium suffruticosum (Ging), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. All the three extract were stored in screw cap vial at 4°C until further use.

B. Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus for 24 hours. Then the marc was subjected to Ethyl acetate (76-78°C) for 24 hrs and then marc was subjected to Methanol for 24 hours. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

C. Animals

Adult male Wistar rats, weighing approximately 150-180g were obtained from Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 per cage, with relative humidity (55%) in a 12 hour light/dark cycle at 25°C±2°C. They were given access to water and a commercial diet ad libitum. The experiment were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.
(CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/CPCSEA/745).

D. Animal diet

The compositions of the two diets were used as follows [14]:

**Control diet:** Wheat flour 22.5%, roasted bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin & choline mixture 0.5%.

**High fat diet:** Wheat flour 20.5%, roasted bengal gram 52.6%, skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt mixture with starch 4% and vitamin & choline mixture 0.5%, cholesterol 0.4%.

E. Experimental Design

A total number of 36 rats were divided into six groups of six rats each:

- **Group I:** Standard chow diet (Control).
- **Group II:** High Fat Diet (HFD).
- **Group III:** HFD + Pet.ether extract of *Ionidium suffruticosum* (250mg/kg B.Wt).
- **Group IV:** HFD + Ethyl acetate extract of *Ionidium suffruticosum* (250mg/kg B.Wt).
- **Group V:** HFD + Methanolic extract of *Ionidium suffruticosum* (250mg/kg B.Wt).
- **Group VI:** HFD + standard drug atorvastatin (1.2 mg/kg body weight)

Both the extracts and atorvastatin were suspended in 2% tween 80 [15] separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the rats were sacrificed by cervical dislocation after overnight fasting. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anaesthesia and blood sample collected in heparinised tubes and plasma was separated. Liver, heart and aorta were cleared of adhering fat, weighed accurately and used for the preparation of homogenate. Animals were given enough care as per the Animal Ethical Committee’s recommendations.

F. Biochemical analysis

Plasma samples were analyzed for total cholesterol, HDL-cholesterol and triglycerides using Boehringer Mannheim kits by Erba Smart Lab analyzer USA. LDL-cholesterol and VLDL-cholesterol were calculated by using Friedwald method [16]. Ester cholesterol [17] and free cholesterol [17] were analyzed by using digitonin. Portions of liver, heart and aorta tissues were blotted, weighed and homogenized with methanol (3 volumes) and the lipid extracts were obtained by the method [18]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides [19], and phospholipids [20]. Free fatty acids were estimated by using method [21]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to access the atherogenic risk.

G. Statistical Analysis

Results were expressed as mean ± SE of 6 rats in each group. The statistical significance between the groups was analysed by using one way analysis of variance (ANOVA), followed by Dunnet’s multiple comparison test. Significance level was fixed at 0.05.

III. RESULTS AND DISCUSSION

Table I illustrates the average body weight changes in control and experimental rats. The body weight of group II rats were increased significantly (p<0.001) in comparison with normal control group I rats. The increment in the weight was reduced significantly (p<0.001) by the administration of methanolic extract of *Ionidium suffruticosum* (250mg/kg) as well as atorvastatin 1.2mg/kg in comparison with the HFD fed rats (group II). The weight reducing effect may be attributed to its potential to inhibit lipogenesis and enhanced thermogenesis, since obesity is associated with defective thermogenesis [22].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Average Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>145±1.82&lt;sup&gt;aNS&lt;/sup&gt;</td>
<td>166.66±2.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.00±3.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>150±2.88&lt;sup&gt;aNS&lt;/sup&gt;</td>
<td>220.00±2.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.00±6.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>145±1.29&lt;sup&gt;aNS, bNS&lt;/sup&gt;</td>
<td>205.83±3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.00±3.87&lt;sup&gt;aNS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>150.83±2.01&lt;sup&gt;aNS, bNS&lt;/sup&gt;</td>
<td>200.00±3.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.00±3.74&lt;sup&gt;aNS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>154.16±1.53&lt;sup&gt;aNS, bNS&lt;/sup&gt;</td>
<td>179.16±2.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.00±4.30&lt;sup&gt;aNS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group VI</td>
<td>156.66±2.74&lt;sup&gt;aNS, bNS&lt;/sup&gt;</td>
<td>174.83±1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.80±3.39&lt;sup&gt;aNS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=6 rats)

*P* values : *<0.001, *<0.05

NS : Non significant

a →group I compared with groups II, III, IV, V, VI.
b →group II compared with groups III, IV, V, VI.

Table II were shown the lipid profile of control and experimental rats. HFD fed rats (group I) had a significant increase (p<0.001) in the total cholesterol and free fatty acid level compared to control rats (group I). Earlier studies reveal significant elevation of lipid parameters in plasma and tissue response to atherogenic diet or high fat diet [23], [24]. Treatment of methanolic extract of *Ionidium suffruticosum* at the dose 250mg/kg body weight (Group V) was found significant (p<0.001) decrease in the total cholesterol and free fatty acid level compared to HFD rats (group II). However, group V showed that the plasma cholesterol and free fatty acid level was restored to near normal as that of group VI (atorvastatin 1.2mg/kg with HFD).
**Table II**

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/dl)</th>
<th>FC (mg/dl)</th>
<th>EC (mg/dl)</th>
<th>FC (mg/dl)</th>
<th>PL (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>A.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>122±5</td>
<td>28±5</td>
<td>94.9±5</td>
<td>40±5</td>
<td>99.5±5</td>
<td>59.2±5</td>
<td>2.0±5</td>
</tr>
<tr>
<td>Gr II</td>
<td>38±5</td>
<td>63±5</td>
<td>1±5</td>
<td>86±5</td>
<td>6±5</td>
<td>0±5</td>
<td>0.05±5</td>
</tr>
<tr>
<td>Gr III</td>
<td>178.4±5</td>
<td>47±5</td>
<td>13.2±5</td>
<td>60±5</td>
<td>13.8±5</td>
<td>72.4±5</td>
<td>4.0±5</td>
</tr>
<tr>
<td>Gr IV</td>
<td>2.59±5</td>
<td>0.1±5</td>
<td>0.92±5</td>
<td>0.1±5</td>
<td>0.26±5</td>
<td>0.6±5</td>
<td>0.0±5</td>
</tr>
<tr>
<td>Gr V</td>
<td>14.2±5</td>
<td>62±5</td>
<td>35±5</td>
<td>19±5</td>
<td>34±5</td>
<td>14±5</td>
<td>0.6±5</td>
</tr>
<tr>
<td>Gr VI</td>
<td>1.88±5</td>
<td>0.5±5</td>
<td>1.21±5</td>
<td>0.1±5</td>
<td>0.11±5</td>
<td>0.2±5</td>
<td>0.0±5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=6 rats). P values: * < 0.001, ** < 0.05, NS: Non Significant

**Table III**

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>LDL/HDL ratio</th>
<th>HDL/c/TC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>59.0±5</td>
<td>24.8±5</td>
<td>11.9±5</td>
<td>0.41±5</td>
<td>0.47±5</td>
</tr>
<tr>
<td>Gr II</td>
<td>38.9±5</td>
<td>42.6±5</td>
<td>14.7±5</td>
<td>0.17±5</td>
<td>0.21±5</td>
</tr>
<tr>
<td>Gr III</td>
<td>42.5±5</td>
<td>34.0±5</td>
<td>13.0±5</td>
<td>0.06±5</td>
<td>0.29±5</td>
</tr>
<tr>
<td>Gr IV</td>
<td>48.6±5</td>
<td>24.1±5</td>
<td>12.0±5</td>
<td>0.03±5</td>
<td>0.36±5</td>
</tr>
<tr>
<td>Gr V</td>
<td>58.7±5</td>
<td>21.7±5</td>
<td>10.7±5</td>
<td>0.02±5</td>
<td>0.51±5</td>
</tr>
<tr>
<td>Gr VI</td>
<td>59.7±5</td>
<td>20.9±5</td>
<td>10.6±5</td>
<td>0.03±5</td>
<td>0.55±5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=6 rats). P values: * < 0.001, ** < 0.05, NS: Non Significant

**Table IV**

<table>
<thead>
<tr>
<th>Ester cholesterol (mg/g tissue)</th>
<th>Liver</th>
<th>Heart</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.68±0.02</td>
<td>1.03±0.04</td>
<td>2.02±0.05</td>
</tr>
<tr>
<td>Group II</td>
<td>0.02±0.01</td>
<td>0.01±0.01</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Group III</td>
<td>3.55±0.02</td>
<td>0.02±0.01</td>
<td>6.81±0.03</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.05±0.02</td>
<td>0.02±0.01</td>
<td>6.34±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=6 rats). P values: * < 0.001, ** < 0.05, NS: Non Significant

**Table V**

<table>
<thead>
<tr>
<th>Triglyceride (mg/g tissue)</th>
<th>Liver</th>
<th>Heart</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.79±0.01</td>
<td>0.73±0.01</td>
<td>0.40±0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>1.28±0.01</td>
<td>1.03±0.03</td>
<td>2.30±0.02</td>
</tr>
<tr>
<td>Group III</td>
<td>1.21±0.01</td>
<td>0.92±0.02</td>
<td>1.42±0.03</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.04±0.01</td>
<td>0.82±0.01</td>
<td>1.11±0.01</td>
</tr>
<tr>
<td>Group V</td>
<td>0.82±0.01</td>
<td>0.62±0.01</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.86±0.04</td>
<td>0.64±0.04</td>
<td>0.63±0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (n=6 rats). P values: * < 0.001, ** < 0.05, NS: Non Significant

As shown in Table II, IV&V. The significant (P<0.001) increase in levels of both free and ester cholesterol were also observed in plasma and tissue of rats fed with high fat diet (group II) when compared to control rats (group I). This high cholesterol concentration in circulation may damage the endothelial cells lining the large arteries and aorta and this may be an initial event in the etiology of atherosclerosis [25]. Both plasma free and ester cholesterol reduced remarkably on treating the HFD rats with methanolic extract of *Iodinium suffruticosum*. This lipid lowering effect may be due to the inhibition of hepatic cholesterologenesis or due to the increase in excretion of fecal sterol [26].
As shown in Table II&VI. The concentration of plasma and tissue triglyceride were increased in rats fed with high fat diet (group II) as compared to control rats (group I). Both plasma and tissue triglyceride levels were significantly decreased in rats treated with methanolic extracts of *Ionidium suffruticosum* at the dose of 250mg/kg and as well as standard drug atorvastatin along with HFD when compared with rats fed with high fat diet (group II). Administration of methanolic extract of *Ionidium suffruticosum* significantly reduced the triglyceride level when compared with other two extract treatment groups. The decrease of plasma triglyceride level is an important finding of this experiment. Recent studies also show that triglycerides are independently related to coronary heart disease [27],[28] and most of the antihypercholesterolemic drugs do not decrease triglycerides when compared to HFD treated rats (group V) might be consequence of increased LDL show an increased risk for cardiovascular diseases [34]. Treatment of methanolic extract of *Ionidium suffruticosum* (Group V) markedly reduced the level of plasma LDL-C and VLDL-C when compared to high fat diet rats (group II). Similar result was not found in other two extract treatment groups. Several studies are shown the extent reduction of CHD incidence is directly related to the magnitude of reduction in LDL-C and VLDL-C levels [35].

As shown in Table III. Elevated levels of LDL and VLDL-cholesterol in rats fed with HFD (group II) was significant (P<0.001) in comparison with control rats (group I). Clinical and epidemiological studies have proved that individuals with elevated LDL show an increased risk for cardiovascular diseases [34]. Treatment of methanolic extract of *Ionidium suffruticosum* (Group V) markedly reduced the level of plasma LDL-C and VLDL-C when compared to high fat diet rats (group II). Similar result was not found in other two extract treatment groups. Several studies are shown the extent reduction of CHD incidence is directly related to the magnitude of reduction in LDL-C and VLDL-C levels [35].

As shown in Table III. The high fat diet rats caused significant (P<0.001) increase in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol. A significant increased in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol indicates increased risk of atherosclerosis and coronary heart disease [36]. Administration of methanolic extract of *Ionidium suffruticosum* along with HFD was found significantly reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol when compared to HFD group (II). An significantly (P<0.001) reduced ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol were observed in methanolic extract of *Ionidium suffruticosum* treated rats (group V) might be consequence of higher proportion of HDL-cholesterol which reduced risk by
voltage of increased reverse cholesterol transport from peripheral organs to liver [37].

Atherogenic index is used as a marker to assess the susceptibility of atherogenesis. As shown in Table 2. In the HFD group, there was a significant increase in the value of atherogenic index 4.57±0.03 (p<0.001), while the group receiving methanolic extract of Ionidium suffruticosum along with high fat diet, showed a significant decrease in atherogenic index 1.92±0.01 (p<0.001), comparable to the control rats (group I) 2.05±0.03(p<0.001). The reduced atherogenic index is inversely related with coronary heart disease and its elevation in considered as an antiatherosclerotic factor [38].

IV. CONCLUSION

The methanolic extract of Ionidium suffruticosum was significantly improved the plasma lipid and lipoprotein profile, thus improving the atherogenic index. It also the significantly reduced the tissues free cholesterol, ester cholesterol, triglycerides and phospholipoids when compared to other extracts. This finding provides some biochemical basis for the use of methanolic extract of whole plant of Ionidium suffruticosum as anti hypertlipidemic agent having preventive and curative effect against hyperlipidemia. Further, studies are required to again more insight in to the possible mechanism of action.

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REFERENCES


