EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF COUMARIN DERIVATIVES ISOLATED FROM *IONIDIUM SUFFRUTICOSUM* IN RATS FED WITH HIGH FAT DIET

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Abstract— The objective of the present study was to investigate the hypolipidemic effect of Coumarin derivatives isolated from Ionidium *suffruticosum* in rat fed with high fat diet. The acute toxicity study was found that Coumarin derivatives isolated from *Ionidium suffruticosum* are safe up to 100mg/kg, so one tenth of this dose (10mg/kg) was consider as evaluation dose. There was a noticed increase in the body weight in HFD fed group (II), which was significantly ($p<0.001$) reduced by the administration coumarin derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg b.wt/day along with high fat diet. High fat diet group of rats showed significant ($p<0.001$) elevation in plasma and tissue total cholesterol, ester & free cholesterol, phospholipids, triglycerides, plasma low-density lipoprotein, and very low-density lipoprotein and lowered HDL cholesterol. Administration of coumarin derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg b.wt/day along with high fat diet significantly ($p<0.001$) reduced in the plasma and tissue total cholesterol , ester cholesterol, triglycerides and phospholipids ,plasma LDL cholesterol and increase the HDL cholesterol when compared to HFD rats. So, the coumarin derivatives isolated from *Ionidium suffruticosum* has definite cardio protective effect against hyperlipidemia.

Keywords— Coumarin derivatives, Hypolipidemic effect, High fat diet, Rats.

I. INTRODUCTION

Cardiovascular disease is a major problem worldwide. The World Health Organization estimates that this disease is responsible for the deaths of approximately 30,000 people each day[1]. Hyperlipidaemia is characterized by elevated serum total cholesterol, low-density lipoprotein cholesterol, very lowdensity lipoprotein cholesterol and decreased high-density lipoprotein cholesterol levels [2] . As reported in literature synthetic drugs may be having serious side effects[3]. Statins may also be associated with some other rarely occurring side effects like nausea, abdominal pain, dyspepsia, diarrhoea or constipation and flatulence [4]. Medicinal plants, on the other hand, have been reported safer as compared topharmaceutically derived remedies[2]. *Ionidium suffruticosum* (Ging.) it belongs to the family Violaceae, it is widely used as traditional healers for the treatment of diseases like diabetes[5] , male sterility [6], urinary tract infections and water retention[7] . Hence, the attempt is made for the evaluation of coumarin derivatives isolated from *Ionidium suffruticosum* for hypolipidemic activity in rats fed with high fat diet.

II. MATERIALS AND METHODS

Collection and identification of plant materials

The whole plant of *Ionidium suffruticosum* (Ging) were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. The taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The *Ionidium suffruticosum* (Ging) were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container.

Preparation of Various extracts from *Ionidium suffruticosum*

The *Ionidium suffruticosum* (Ging) were dried in shade and powdered. The powdered plant materials were successively extracted with petroleum ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus[8] (Harborne 1984) for 24 hrs. Then the marc was dried and then subjected to ethyl acetate (76-78°C) for 24 hrs, then marc was dried and then it was subjected to methanol (80ºC) for 24 hrs. The solvent from the extracts was recovered under reduced pressure using rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Isolation of Coumarin derivatives from methanolic extract of *Ionidium suffruticosum*

The various extract of *Ionidium suffruticosum* were evaluated the anti atherogenic and antioxidant activities . The methanolic extract of *Ionidium suffruticosum* was providing better result than that of other two extracts. Therefore, the methanol extract of *Ionidium suffruticosum* was subjected to the TLC chromatographic profile and column chromatographic separation. The methanolic extract of *Ionidium suffruticosum* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g methanolic extract of *Ionidium suffruticosum*) was adsorbed on

silica gel (20 g) and transferred to a column of silica gel (200g equilibrated with benzene). The Coumarin derivative (245 mg) was eluted with benzene: Chloroform 70:30, v/v from methanolic extract of *Ionidium suffruticosum.*

Experimental Animals

Healthy male Wistar rats (weighing 120-150g) were procured 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 $25^{\circ}\pm2^{\circ}$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).

Acute toxicity studies

Acute oral toxicity study was performed as per Organization and Economic Cooperation and Development (OECD) guidelines 423 adopted on 17th December 2001 received from Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA). The rats were fed with Coumarin derivatives from *Ionidium suffruticosum* suspended in 1% gum acacia at the dose of 100mg/kg body weight. The animals were observed individually every 30 minutes after dosing the first 24hrs and thereafter daily for a total of 14 days. The time at which signs of toxicity appear and disappear was observed systematically and recorded for each animal.

Experimental Induction of Hyperlipidemia

High fat diet was prepared by mixing 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%[9] . The diet was placed in the cage carefully and was administered for 14 days [10].

Protocol for Antihyperlipidemic Activity

In the experiment a total number of 24 rats were used. The rats were divided into four groups of six each.

Group I : Standard chow diet (Control)
Group II : High Fat Diet
Group III : High fat diet + Coumarin derivative from *Ionidium suffruticosum* (10mg/kg b.wt)
Group IV : High fat diet + standard drug atorvastatin (1.2 mg/kg B.wt)

Both the isolated compounds and atorvastatin were suspended in 2% tween 80 [11](Waynforth 1980) separately and fed to the respective rats by oral intubation. At the end of the study 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.

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 [12]. Ester cholesterol [13] and free cholesterol[13] 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 of Folch et al.1957[14]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides[15] and phospholipids [16]. The atherogenic Index was calculated by using the following formula.

$$\text{Athrogenic Index} = \frac{Totalcholesterol}{HDL}$$

III. STATISTICAL ANALYSIS

Results were expressed as mean ± SE of 6 rats in each group. The data were also analysed by one way analysis of variance (ANOVA) followed by Dunnet's t-test. 'P, value less than 0.05 is considered significant.

IV. RESULTS AND DISCUSSION

Balance between synthesis and degradation of biological tissues is maintained by Lipid metabolism. Development of hyperlipidemia disease is a complicated process involving accumulation of lipid containing particles in the walls of coronary arteries & other major arteries within the body. A high-fat diet causes cholesterol levels to increase in susceptible people, which leads to obesity.

The coumarin derivatives isolated from *Ionidium suffruticosum* were found to be non-toxic upto the dose of 100mg/kg and did not cause any death of the tested animals. Therefore, one tenth of this dose (10mg/kg) was considered as the evaluation dose. As shown in Table 1. The weight gain in HFD group of rats was significantly ($p<0.001$) higher than control rats reflecting the influence of high fat diet. The increment in the weight was reduced significantly ($p<0.001$) by the administration of Coumarin derivatives isolated from *Ionidium suffruticosum* (Group III) at the dose of 10mg/kg as well as atorvastatin 1.2mg/kg in comparison with the HFD

fed rats (Group II). However, treatment with Coumarin derivatives isolated from *Ionidium suffruticosum* (10mg/kg) exhibited the significant reduction in bodyweight in comparison with the group received 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[17] .

As shown in Table 2. High fat diet fed rats showed significant increase ($p<0.001$) in plasma total cholesterol level as 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[18] . Treatment with coumarin derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg body weight had showed a significant ($p<0.001$) decrease in the total cholesterol level compared to HFD rats (group II). Lowering high cholesterol levels significantly reduced the risk of heart attacks, strokes, and death. However, the rats received coumarins derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg b.wt (Group III) with HFD had showed restored the plasma cholesterol level near to normal as that of standard (Group IV).

As shown in Table 2 &3. 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 [19]. Both plasma free and ester cholesterol reduced remarkably on treating the HFD rats with coumarin derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg body weight. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of fecal sterol [20].

As shown in Table 2&4. The concentration of plasma and tissue triglyceride was elevated in rats fed with high fat diet (group II) as compared to control rats (group I). HFD rats had significant increase in the level of plasma triglyceride due to decrease in the activity of lipoprotein lipase[21] . Both plasma and tissue triglyceride levels were significantly reduced in rats treated with at the dose of 10mg/kg body weight, coumarin derivatives isolated from *Ionidium suffruticosum* at the dose of 10mg/kg body weight and as well as standard drug atorvastatin along with HFD when compared with rats fed with high fat diet (group II). The coumarin derivatives isolated from *Ionidium suffruticosum* may have stimulation of lipoprotein lipase activities resulting in decrease of plasma triglyceride and might increase the uptake of triglyceride from plasma by skeletal muscle and adipose tissues [22].

As shown in Table 2 &5. The concentration of plasma and tissue phospholipids were significantly increased in rats fed HFD (group II) as compared to control animals (group I). This may be due to decreased phospolipase activity[23] . Both plasma and tissue phospholipids levels were significantly decreased in rats treated with coumarin derivatives isolated from *Ionidium suffruticosum* (10mg/kg body weight) and as well as standard drug atorvastatin along with HFD when compared with rats fed with high fat diet (group II). The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases[10] .

Atherogenic Index (AI) indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney. The higher the AI, higher is the risk of above organs for oxidative damage. As shown in Table 2. The atherogenic index (TG/HDL-C ratio) used to predict risk of CHD and marker of small, dense LDL-C (an atherogenic lipoprotein)[24] were significantly reduced by the coumarin derivatives isolated from *I.suffruticosum*, indicating the beneficial effect of flavones and coumarins in cardiovascular diseases.

As shown in Table 6. The reduction in the HDL produced by the group of animals fed with HFD was significant ($P<0.001$) in comparison with group I animals. This result is highly significant in that low HDL-cholesterol is now considered as the most significant risk factor for atherosclerosis [25] . However, the treatment with coumarin derivatives isolated from *Ionidium suffruticosum* (Group III) had significantly increased the HDL-cholesterol level when compared to HFD rats (Group II). It has clearly been demonstrated that a relationship exists between increased concentration of HDL-C and decreased morbidity-and mortality-rate in cardiovascular patients[26] .

As shown in Table 7. The 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). High cholesterol diet increased serum cholesterol and LDL-C level significantly. Clinical and epidemiological studies have proved that individuals with elevated LDL show an increased risk for cardiovascular diseases[27] . Treatment of coumarin derivatives from *Ionidium suffruticosum* (Group III) markedly reduced the level of plasma LDL-cholesterol and VLDL-cholesterol when compared to HFD rats (group II). Recent research has revealed that a 4-5% decrease in LDL-cholesterol results in a 5-10% decrease in the occurrence of coronary heart disease (CHD) [28].

Table 1: Average Body weight changes in control and experimental rats

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	137.34 ± 0.47^{bNS}	$156.66\pm2.47^{b*}$	$19.32\pm2.39^{b*}$
Group II	136.33 ± 0.42^{aNS}	$198.64\pm2.58^{a*}$	$62.31\pm1.90^{a*}$
Group III	$136.57\pm0.53^{aNS,bNS}$	$161.26\pm1.38^{aNS,b*}$	$24.69\pm3.28^{aNS,b*}$
Group IV	$137.55\pm0.74^{aNS,bNS}$	$161.51\pm1.74^{aNS,b*}$	$23.96\pm3.39^{aNS,b*}$

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.;b → group (Negative Control).;Group III : HFD+Coumarin derivatives isolated from *Ionidium suffruticosum* (10mg/kg b.wt);Group IV : HFD + Atorvastatin (1.2 mg/kg b.wt) (Positive Control).

Table 2: Effect of Coumarin derivatives from *Ionidium suffruticosum* on plasma lipid profile in control and experimental rats

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	$69.81\pm 0.51^{b*}$	$23.67\pm 0.43^{b*}$	$46.14\pm 0.61^{b*}$	$155.87\pm 0.24^{b*}$	$78.57\pm 0.20^{b*}$	$2.90\pm 0.08^{b*}$
Group II	$167.17\pm 0.26^{a*}$	$49.41\pm 0.12^{a*}$	$117.76\pm 0.57^{a*}$	$208.33\pm 0.33^{a*}$	$121.83\pm 0.33^{a*}$	$9.73\pm 0.21^{a*}$
Group III	$72.74\pm 0.47^{a*,b*}$	$23.05\pm 0.40^{a*,b*}$	$49.69\pm 0.22^{a*,b*}$	$156.47\pm 0.12^{a*,b*}$	$79.47\pm 0.37^{a*,b*}$	$2.88\pm 0.84^{a*,b*}$
Group IV	$72.92\pm 0.22^{a*,b*}$	$23.44\pm 0.55^{a*,b*}$	$49.48\pm 0.56^{a*,b*}$	$156.29\pm 0.28^{a*,b*}$	$80.19\pm 0.07^{a*,b*}$	$2.97\pm 0.53^{a*,b*}$

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.
b → group II compared with groups III, IV;Details of group I-IV are same as in Table 1.

Table 3: Effect of Coumarin derivatives from *Ionidium suffruticosum* on tissues ester and free cholesterol profile in control and experimental rats

Groups	Ester cholesterol (mg/g tissue)			Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta	Liver	Heart	Aorta
Group I	$0.67\pm 0.02^{b*}$	$2.86\pm 0.01^{b*}$	$2.02\pm0.42^{b*}$	$0.65\pm0.01^{b*}$	$0.69\pm 0.01^{b*}$	$0.40\pm0.01^{b*}$
Group II	$2.55\pm0.04^{a*}$	$6.96\pm0.02^{a*}$	$6.81\pm0.23^{a*}$	$1.24\pm0.01^{a*}$	$1.17\pm0.03^{a*}$	$1.86\pm0.02^{a*}$
Group III	$2.59\pm0.03^{a*,b*}$	$3.10\pm0.01^{a*,b*}$	$2.69\pm0.09^{a*,b*}$	$0.73\pm0.01^{a*,b*}$	$0.66\pm0.01^{a*,b*}$	$0.62\pm0.01^{a*,b*}$
Group IV	$1.84\pm0.01^{a*,b*}$	$2.98\pm0.01^{a*,b*}$	$2.83\pm0.11^{a*,b*}$	$0.81\pm0.04^{a*,b*}$	$0.64\pm0.04^{a*,b*}$	$0.63\pm0.04^{a*,b*}$

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.
b →group II compared with groups III, IV;Details of group I-V are same as in Table 1.

Table 4: Effect of Coumarin derivatives from *Ionidium suffruticosum* on tissues Triglyceride level in control and experimental rats

Groups	Triglyceride (mg/g tissue)		
	Liver	Heart	Aorta
Group I	$7.95\pm0.01^{b*}$	$9.17\pm0.01^{b*}$	$9.48\pm0.33^{b*}$
Group II	$18.11\pm0.19^{a*}$	$28.79\pm0.16^{a*}$	$19.37\pm0.78^{a*}$
Group IV	$8.24\pm0.10^{a*,b*}$	$15.47\pm0.12^{a*,b*}$	$12.08\pm0.12^{a*,b*}$
Group V	$8.31\pm0.69^{a*,b*}$	$15.21\pm0.36^{a*,b*}$	$12.15\pm0.01^{a*,b*}$

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.
b →group II compared with groups III, IV; Details of group I-V are same as in Table 1.

Table5: Effect of Coumarin derivatives from *Ionidium suffruticosum* on tissues Phospholipids level in control and experimental rats

Groups	Phospholipids (mg/g tissue)		
	Liver	Heart	Aorta
Group I	$12.37\pm0.45^{b*}$	$18.04\pm0.67^{b*}$	$10.32\pm0.05^{b*}$
Group II	$25.14\pm0.69^{a*}$	$26.47\pm0.10^{a*}$	$17.45\pm0.29^{a*}$
Group III	$16.45\pm0.06^{a*,b*}$	$19.45\pm0.73^{a*,b*}$	$12.47\pm0.12^{a*,b*}$
Group IV	$17.45\pm0.77^{a*,b*}$	$19.08\pm0.18^{a*,b**}$	$12.02\pm0.10^{a*,b*}$

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.
b →group II compared with groups III, IV.;Details of group I-V are same as in Table 1.

Table 6: Effect of Coumarin derivatives from *Ionidium suffruticosum* on plasma lipoprotein in control and experimental rats

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Group I	24.04±0.16^{b*}	30.06±0.41^{b*}	15.71±0.10^{b*}
Group II	17.17+0.22^{a*}	125.64+0.55^{a*}	24.36+0.17^{a*}
Group III	25.27±0.19^{a*,b*}	31.58± 0.08^{a*,b*}	15.89±0.18^{a**,b*}
Group IV	24.51±0.26^{a*,b*}	32.37± 0.19^{a*,b*}	16.04±0.03^{a*,b*}

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.
b →group II compared with groups III, IV.;Details of group I-V are same as in Table 1.

CONCLUSION

On the basis of the present investigation was observed as the Coumarin derivatives isolated from *Ionidium suffruticosum* had showed a better antihyperlipidemic activity in comparison with HFD group of rats . The Coumarin derivatives isolated from *Ionidium suffruticosum* is beneficial in preventing atherosclerosis and reducing risk factors for coronary artery disease.

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