Research Article

Evaluation of Hypolipidemic Activity of Ethanolic and Aqueous Extracts of *Fragaria vesca* in High Fat Diet Induced Hyperlipidemia in Rats

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ABSTRACT

Dyslipidemia, an important risk factor contributing to atherosclerosis, Ischemic heart diseases & Cerebrovascular accidents. Currently used hypolipidemics are not free of adverse effects. Hence research is required to find better agents which are equally effective with less adverse effects. To evaluate the hypolipidemic activity of ethanolic (EEFV) & aqueous (AEFV) extracts of Fragaria Vesca in high fat diet induced hyperlipidemia in Wistar rats. 36 rats weighing 180-200gm were divided into 6 groups with six in each & these were fed with high fat diet for one month. After establishing hyperlipidemia, animals were allocated to the following treatment groups. Group I - control, Group II treated with standard drug, Atorvastatin, group III & IV with ethanolic extract 250 & 500 mg/kg respectively and group V & VI with aqueous extract 250 & 500 mg/kg respectively. Atorvastatin & test drug were given per oral for 4weeks. Serum lipid profile, blood glucose & body weight were estimated on day 0, 30 & 60 and the results were analyzed by ANOVA. Significant reduction in total cholesterol, triglyceride, LDL & VLDL in all groups except in control & group V. HDL level was increased in group II, III, IV. Blood glucose was not significantly changed. Body weight was significantly increased in all groups after high fat diet & decreased after treatment. Ethanolic extract (250,500mg) and aqueous extract (500mg/dl) of Fragaria vesca have significant hypolipidemic activity and the effect of ethanolic 500 mg/kg was almost equal to Atorvastatin.

Keywords: Ethanolic and aqueous extracts, Fragaria vesca, High fat diet, Hyperlipidemia, Hypolipidemia Atorvastatin, Lipid profile.

INTRODUCTION

Dyslipidemia is an important risk factor contributing to Ischemic heart disease and stroke, which have has been reported as the most common cause of death in developed as well as developing countries.

"Globally, a third of ischaemic heart disease is attributable to high cholesterol. Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYS), or 2.0% of total DALYS" [1]

Hence hypolipidemic drugs are extensively used both for prophylaxis & treatment of hyperlipidemia. Currently used hypolipidemic agents such as statins and fibrates, though extensively used all over the world for more than a decade, are not free of adverse effects. Myopathy & rhabdomyolysis are well known adverse effects of statins & fibrates where as statins can aggravate / cause Pancreatitis.[2] Recently statins have also been reported to increase the risk of raised blood sugar levels and the development of Type 2 diabetes[3] and FDA has given a warning to this effect.

Under these circumstances it is relevant to evaluate the medicinal plants which are reported to have hypolipidemic activity at the same time devoid of adverse effects. Results of a meta-analysis done by Hasani-Ranjbar S et al 2010 showed that natural products were found effective in the treatment of hyperlipidemia.[4]

The advantages of herbal medicines reported are effectiveness, safety, affordability and acceptability.[5]

More than 100 medicinal plants have been documented to have significant hypolipidemic action.

Fragaria vesca (FV) is one of the medicinal plants consumed by humans. In the traditional system of medicine, the plant is used as blood purifier. Fruits of this plant are reported to possess many medicinal properties.

Fragaria Vesca is also known as wild strawberry, Alpine Strawberry, European Strawberry, Woodland Strawberry & belongs to the family of Rosacea, native to Northern Hemisphere.

Phytochemical composition

FV contains phenolic compounds mainly flavonoids (anthocyanins, with flavonols), hydrolyzable tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), with condensed tannins (proanthocyanidins).[6]

Nutritional value

Contains dietary fiber, fructose carotenoids and tocopherols, vitamin C, folate and minerals like Calcium, Iron, Magnesium, Phosphorus, Potassium, Sodium, Zinc, Copper, Manganese & Selenium. The seed oil is rich in unsaturated fatty acids.

Medicinal use

Traditionally strawberry is used for a wide range of conditions including diarrhoea, liver diseases, inflammatory conditions, respiratory tract infections, gout, arthritis, nervous tension, oedema, renal stones, fever, night sweats and anaemia.

It is also used for purifying the blood, stimulating metabolism, preventing menstruation and supporting natural weight loss. Strawberry wrapped in a cloth and used as compress for skin rashes.[7]

Studies conducted in the current decade have shown that FV has analgesic[8], diuretic & nephroprotective[9], anticonvulsant[10], anti-inflammatory[11], hypolipidemic[12] & antibacterial activity.[13] Among these activities most of them have been exhibited by the fruit or the whole plant. The hypolipidemic activity has been reported for the leaves. Whether the fruit has hypolipidemic activity is not known. As the fruit is commonly consumed, the current study was undertaken to evaluate the hypolipidemic activity of fruit pulp extracts of Fragaria vesca in high diet induced hyperlipidemia in rats.

Objectives

To find out whether the fruit pulp extracts of FV reduce lipid levels in rats

Secondary objective

To assess its action blood sugar level & body weight

MATERIALS AND METHODS

The study was started after getting approval from Institutional Animal Ethics Committee. Approval No. IAEC 2/Desp.No.51/Dt.29.07.13.

Animals

Wistar albino rats weighing between 180 - 200 g were procured from "Central Animal House, Chettinad Hospitals and Research Institute", Chennai, India. The animal house was well ventilated and the animals had 12 ± 1 h day and night rhythm throughout the experimental period. The animals were housed in large spacious polypropylene cages. Guidelines for breeding and Experiments on Animals, 1998 defined by the Ministry of Social Justice and Empowerment of India was followed. The animals received a balanced commercially available pelleted rat feed and were provided with clean drinking water.

Diet and Reagents

The high fat diet components such as cholesterol, cholic acid, casein, choline, sucrose was purchased from Himedia Laboratories Pvt. Ltd., Chennai, multivitamin multi mineral capsules Becadexamin and Atorvastatin were obtained from institutional pharmacy. Diagnostic kits for the estimation of TC, triglyceride and HDL-C will be obtained from Coral Ltd., Goa.

Plant Material

Pulp of Fragaria vesca fruit extract was used for this study.

Fragaria vesca fruits were collected from local market of Chennai. The fresh fruits were washed and cleaned with water to remove dirt, seeds removed &chopped, shade dried and pulverized.

Preparation of Extracts

Pulverized fruits were extracted in Soxhlet apparatus with ethanol. Aqueous extract was prepared by decoction. The extracts were filtered and the filtrate obtained was evaporated to dryness by vacuum evaporator.

Induction of hyperlipidemia

Rats were divided into 6 groups of 6 animals each. The animals of all the groups were given a high fat diet consisting of cholesterol (1%), cholic acid (0.5%), casein (20%), choline (0.25%), d-l-methionin1(0.4%), coconut oil (25%), multi vitamin mix (3.5%) and sucrose (48.4%) with standard pellet diet for 30 days.

Methodology

Basal lipid profile, body weight & blood sugar were estimated for all the animals before giving high fat diet. High fat diet prepared was in the pellet form & mixed with normal pellet diet & given 30 days to produce hyperlipidemia. The cages were checked daily & confirmed that the animals had consumed the high fat pellet. The average basal total cholesterol was 67-78mg/dl. Rats having total cholesterol more than 95 mg/dl were included in the study. They were divided into six groups as given below.

Experimental design

Group 1: High fat diet

Group 2: High fat diet +Standard drug Atorvastatin (10mg/kg) per oral for 4 weeks

Group 3: High fat diet + Ethanolic extract of fruit fragaria vesca (EEFV) 250mg/kg per oral for 4 weeks

Group 4: High fat diet + Ethanolic extract of fruit fragaria vesca (EEFV) 500mg/kg per oral for 4 weeks

Group 5: High fat diet + Aqueous extract of fragaria vesca (AEFV) 250mg/kg per oral for 4 weeks

Group 6: High fat diet + Aqueous extract of fragaria vesca (AEFV) 500mg/kg per oral for 4 weeks

Collection of Blood

Blood was collected by retro orbital sinus puncture, under mild halothane anaesthesia. The collected samples were centrifuged for 10 minutes at 2000 r.p.m. and serum samples so collected were used for various biochemical tests.

Estimation of Blood sugar

Blood glucose levels were estimated using an electronic glucometer (Accu Chek Active Glucometer - mg dL-1).

Lipid profile

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were measured using standard diagnostic kits supplied by SPAN diagnostics, India. LDL-cholesterol and VLDL-cholesterol were calculated by Friedwald formula.[14]

Statistical Analysis

Values were expressed as Mean ±SEM for six rats per group and analyzed by using one way ANOVA followed by Dunnets test. $p < 0.05$ was considered significant

RESULTS

Lipid profile

All the groups supplemented with high fat diet (HFD) showed significant increase ($p<0.05$) in serum total cholesterol, Triglyceride & LDL-C, but significant decrease ($p<0.05$) in HDL-C level as compared to values before supplementing high fat diet. The serum levels of TC, TG, LDL-c, HDL-c before giving HFD, after supplementing HFD & after treatment with Atorvastatin & extracts of Fragaria vesca was shown in Table-1.

The standard drug Atorvastatin and both the doses of ethanolic extract (EEFV) [250mg/kg, 500 mg/kg] & Aqueous extract (AEFV)[500mg/kg] showed significant reduction ($p<0.05$) in total cholesterol (Figure 1), triglyceride (Figure 2) & LDL-C (Figure 3) but significant increase ($p<0.05$) in HDL-C (Figure 4) as compared to control group. The reduction seen with aqueous 250mg/kg was not found to be statistically not significantly.

Table 1: Lipid profile in all groups before & after treatment

Parameter	BT/ AT	G-1	G- 2	G- 3	G-4	G- 5	G- 6	Compare with G- I To others	Compare with G-2 To other
TC	BT	98.8±10.4	119.5±1.87	110±5.80	120.1±5.2	109.3±7.1	105.8±2.7	GII, GIII, GIV, GV, GVI ***	GI *** GIV ns GV *** GVI *
	AT	139.8±3.9	62.2±5.36	80.5±4.48	76.6±3.0	104.3±5.1	82.1±1.15		
TG	BT	84.3±3.34	118±8.079	111.6±3.7	109.6±5.5	114.5±4. 71	99.8±3.97	GII,GIII,IV,VI *** GV ns	GI *** GIII, GIV, GVI ns GV ***
	AT	112.6±10	72.5±2.75	71.66±3.3	73.2±2.94	102.5±4.54	72.16±2.56		
LDL	BT	57.4±8.32	85.2±2.2	71.0±4.71	79.6±4.15	71.2±5.22	69.76±2.61	GII,GII,IV,V,VI ***	GI ***GIII* GIV ns GV*** GVI *
	AT	101.7±3.7	26.4±4.3	48.8±3.7	44.9±2.27	70.3±.86	49.5±0.97		
HDL	BT	14.66±0.95	10.4±1.07	16.1±0.89	15.33±1.1	15.23±1.07	16.1±0.89	GII,GIV*** GIII,GV&GVI ns	GI *** GIII* GIV ns GV*** GVI *
	AT	15.63±0.51	21.2±1.073	18.1±1.19	20.4±1.04	15.7±1.73	18.1±1.19		

All the values are expressed mean ± SEM, n=6; *p<0.05, **p<0.01, ***p<0.001, ns-not significant

Table 2: Effect of Fragaria vesca on blood glucose in rats fed with high fat diet

Group	Blood glucose (Before treatment)	Blood glucose (after treatment)	Compare with Group-I (HFD) to other groups	Compare with Group –II (STD) to other groups
Group I	88.3+3.36	100.3+5.45	-	* P<0.05
Group II	106+3.99	75.5+3.34	* P<0.05	-
Group III	101+5.07	83.6+2.74	ns P>0.05	ns P>0.05
Group IV	15.33±1.10	24.43±1.04	ns P>0.05	ns P>0.05
Group V	10.23±1.07	24.7±1.073	ns P>0.05	G.II to G.IV ns P>0.05
Group VI	16.1±0.89	19.1±1.19	GI to G.VI NS P>0.05	G.II to G.VI *P<0.05

All the values are expressed as mean ± SEM, n=6; *-p<0.05, ** p<0.01, *** P<0.001, ns- not significant

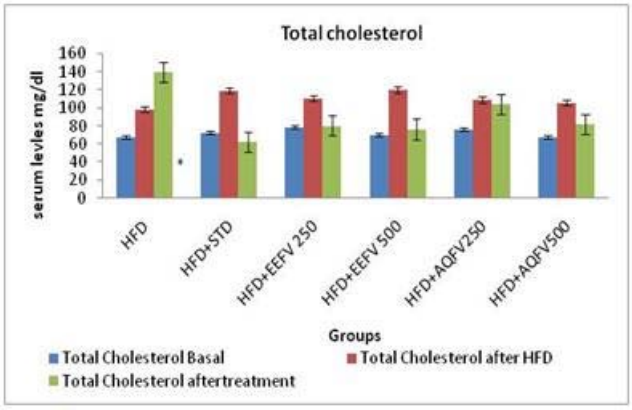
All the groups were compared with control high fat model & Atorvastatin in (Figure 5). The effect on lipid profile by EEFV 500mg/kg was almost equal to standard drug Atorvastatin (Table 1). Lipid profiles for all groups were compared in Figure 5 after treatment.

Serum Glucose

In our study serum glucose level was not significantly changed after high fat diet & also after treatment compare to basal levels as shown in Table 2.

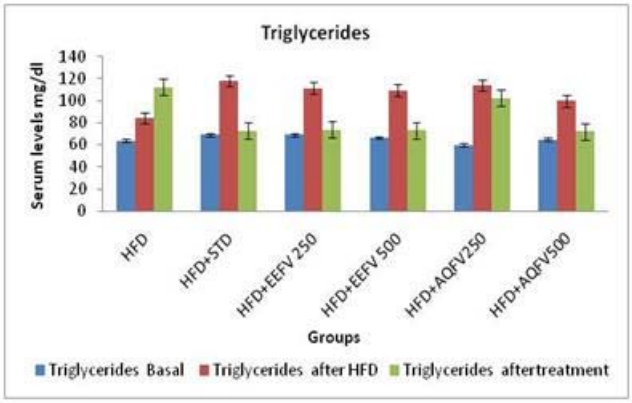
Effect on body weight

Body weight of rats increased after giving high fat diet in all groups. After treatment with Atorvastatin and EEFV [250mg/kg, 500 mg/kg], AQFV [250, 500 mg/kg] the body weight was reduced and there was significant deference between fat model and treated groups (Figure 2). P<0.01 was observed between fat model and standard & EEFV 250mg/kg and p<0.001 for fat model and other groups.



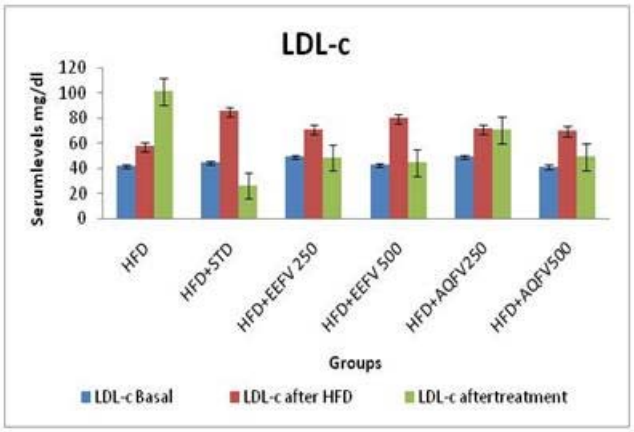
The values were expressed mean ± S.E.M. for six rats

Figure 1: Effect of Fragaria vesca extracts on total cholesterol



The values were expressed mean ± S.E.M. for six rats

Figure 2: Effect of Fragaria vesca extracts on triglycerides

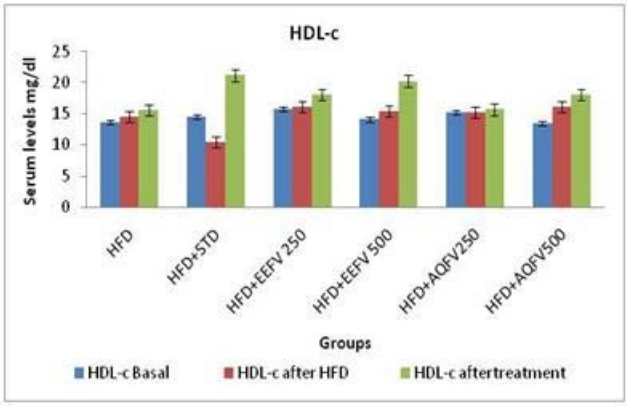


The values were expressed mean ± S.E.M. for six rats

Figure 3: Effect of Fragaria vesca extracts on LDL-c

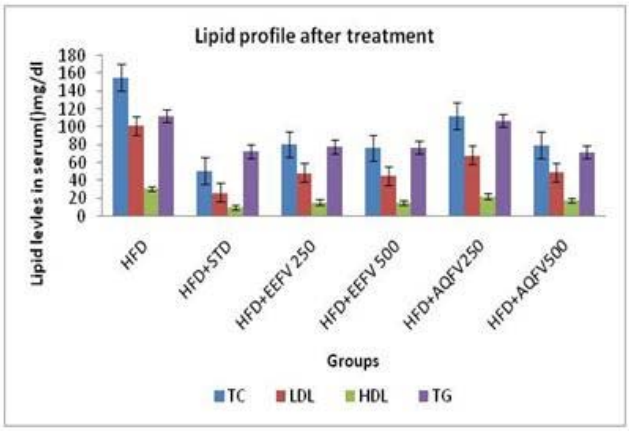
DISCUSSION

Hyperlipidemia has been documented as a major causative factor for atherosclerosis and coronary heart disease. The WHO has estimated that 23.3 million people will die annually from CVD, by 2030. Development of atherosclerotic disease is a complicated process involving accumulation of lipid-containing particles in the walls of arteries causing obstruction and reduced blood supply. High levels of HDL-C & reduced total cholesterol, triglycerides & LDL-c can lower the risk of atherosclerosis & heart disease. The drugs used currently for hyperlipidemia / dyslipidemias are statins, fibrates, niacin & ezetimibe. Statins role in preventing cardiovascular disease has been well established. But recently certain limitations to the use of statins have been observed & FDA has advised the consumers and health care professionals that statins have to be taken with care and knowledge of their side effects such as Cognitive impairment & increased risk of raised blood sugar levels and the development of Type 2 diabetes.[15] Studies have already shown that incidence of statin-related myopathy is up to 9%.[16]



The values were expressed mean ± S.E.M. for six rats

Figure 4: Effect of Fragaria vesca extracts on HDL-c

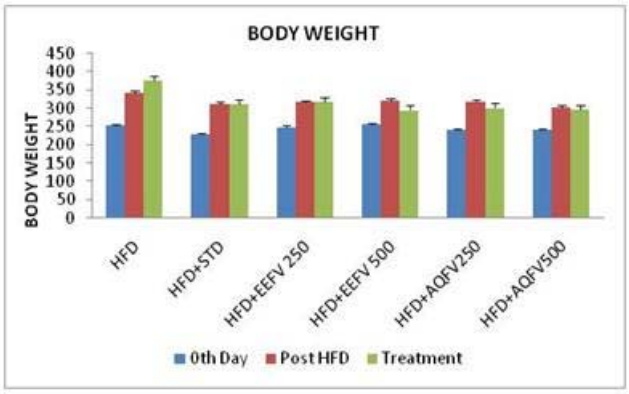


The values were expressed mean ± S.E.M. for six rats

Figure 5: Effect of extract of Fragaria Vesca on serum TC, TGs, LDL-c and HDL-c of rats fed high fat diet in comparison with hyperlipidemia control group

Hence there is a need to find alternative management for hyperlipidemia. Berries such as blueberry, blackberry and strawberry are known as "super fruits" for their nutritional value.[17] Strawberry (Fragaria vesca), among them is a widely consumed one & has been reported to have many medicinal properties. The leaves are found to have hypolipidemic activity. This study was under taken to evaluate the hypolipidemic activity of the fruit extracts of Fragaria Vesca as the fruits are commonly consumed and not the leaves. The hyperlipidemia model used in this

study was the standard model used as it closely resembles human dyslipidemia.



The values were expressed mean ± S.E.M. for six rats

Figure 6: Effect of Fragaria vesca on body weight in rats fed with high fat diet

The results of this study have shown that both the aqueous and ethanolic extracts of the fruits have lipid lowering activity. The ethanolic extract was found to have better hypolipidemic effect than the aqueous extract. The effect of ethanolic extract 500 mg was almost equal to that of Atorvastatin. Increased HDL-c was seen with standard drug & EEFV 500 mg/kg.

All the groups showed an increase in the body weight after giving high fat diet. After treatment with Atorvastatin and ethanolic & aqueous extracts, the body weight was reduced and there was significant difference between control and treated groups.

There was no significant change in the blood glucose levels in all the groups. Though FDA has reported that there may be a risk of diabetes in patients on long term statin therapy, no change the blood glucose was observed in our study, this may be due to the short term administration of Atorvastatin (for 1 month).

Atorvastatin is a HMG-CoA reductase inhibitor and acts by inhibiting the cholesterol synthesis in the liver, reducing the total cholesterol, VLDL & triglycerides. Atorvastatin also reduces LDL by increasing the number of LDL receptors.

Fragaria vesca may act by one of the following mechanisms like inhibiting the cholesterol synthesis in the liver or the absorption of fat from intestine and by decreasing the LDL cholesterol oxidation. Strawberries are rich in vitamin C, flavonoids, phenolic acids and anthocyanins [18] and all these components have high free radical scavenging activity and inhibit LDL-c cholesterol oxiadation.[19] It is reported that, the use of strawberries in the prevention of cancer, cardiovascular and other chronic diseases is due to its antioxidant activity.[20]

Flavonoids have been reported to have hypolipidemic activity.[21] They increase bile acid excretion and reduce cholesterol level in the body.[22] Flavonoids also lower triglycerides & activate LDL receptors which contain 7 to 10% triacyl-glycerol in their structure.[23] The absorption of dietary cholesterol in the distal ileum occurs through solubilisation in the mixed micelles. Flavonoids acts as cofactor of the enzyme cholesterol esterase and inhibit the cholesterol micellar solubility which will result in decreased absorption of dietary cholesterol.[24]

In a recent well-controlled clinical trial, 123 healthy persons who were fed on a diet containing nine servings per day of fruits and vegetables showed significantly increased serum antioxidant capacity and decreased in vivo lipid peroxidation.[25]

Thus flavanoids present in straw berry fruit would have contributed to the hypolipidemic activity by decreasing cholesterol synthesis & absorption of dietary cholesterol. But further studies are required to evaluate the exact mechanism of hypolipidemic action of Fragaria vesca.

CONCLUSION

It can be concluded from the present study that both the ethanolic (250 & 500mg) and aqueous extracts (500mg/kg) of Fragaria vesca have significant hypolipidemic activity. The effect of 500 mg of ethanolic extract was found to be equal to standard drug, Atorvastatin. Both the extracts did not alter the blood glucose level.

The gain in body weight of rats fed on high fat diet was reduced both in the standard and test groups. Hence the preparation of Fragaria vesca extract can be used as alternative hypolipidemic agent in the treatment of hyperlipidemia & cardiovascular diseases especially among diabetics. Further clinical studies are required to use Fragaria vesca as hypolipidemic agent in humans.

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