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Effect of Methanolic Extract of Tuberos Root of *Ipomoea Digitata* (Linn) on Hyperlipidemia induced by rat fed with high fat diet

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ABSTRACT

The aim of the present study was to investigate the effect of the methanolic extract of tuberos root of *Ipomoea digitata* in reducing the cholesterol levels in experimentally induced hyperlipidemic rats. The elevated levels of total cholesterol, ester & free cholesterol, phospholipids, triglycerides, low-density lipoprotein, and very low-density lipoprotein due to HFD. Administration of methanolic extract of *Ipomoea digitata* (300mg/kg) was significantly ($P < 0.001$) reduced the lipid profile and lipoprotein levels. A significant reduction in HDL-cholesterol was noticed in HFD fed groups (II); however, a significant increased the HDL level was produced by the administration of methanolic extract of *Ipomoea digitata* (dose 300mg/kg). There was a noticed increase in the body weight in HFD fed group (II), which was reduced by the administration of methanolic extract of *Ipomoea digitata* (dose 300mg/kg). Therefore, it was concluded that the methanolic extract of tuberos root of *Ipomoea digitata* has definite cardio protective effect against hyperlipidemia.

Key words: *Ipomoea digitata*, hyperlipidemic effect, HFD.

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INTRODUCTION

Atherosclerosis, the most important pathologic process leading to cardio- and cerebrovascular diseases, is suggested to be mediated by the increase in the serum lipid, thrombosis, and injuries of the endothelial cells [1,2]. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol established [3]. Some of the major limitations in the effective pharmacological treatment of hyperlipidemia are the constraints imposed on health care resources, particularly in the low- and middle-income countries [4]. There is a need to tackle this physiological problem as it is attaining grave proportions globally. In this scenario, the problem may be tackled by use of natural agents due to their cost effectiveness and minimal side-effects [5]. In recent times, much research interest has been focused on various herbs that possess hypolipidemic properties that may be useful in reducing the risk of cardiovascular diseases [6].

The tuberous root of *Ipomoea digitata* (Linn) is belongs to the convolvulaceae family. The roots are large ovoid or elongated tuberous roots. The root is regarded as a diuretic, leprosy, burning sensation, vomiting and disease of blood, anthelmintic, syphilis and spleen disease [7]. The plant used as Aphrodisiac activity [8] and anti microbial activity [9]. Resin glycoside was isolated from leaves and stems of *Ipomoea digitata* [10]. Hence, the work was carried out to evaluate the hypolipidemic activity of methanolic extract of tuberous root of *Ipomoea digitata* on rats fed with high fat diet.

MATERIAL AND METHODS

Collection and identification of plant materials

The tuberous root of *Ipomoea digitata* (Linn), were collected from Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The tuberous root of *Ipomoea digitata* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powdered materials were successively extracted with methanol by hot continuous percolation method in Soxhlet apparatus¹¹ for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The extracts were suspended in 2% tween 80 [12].

Animals and treatment

Male Wister rats of 16-19 weeks age, weighing 150-175g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were kept in cages, 2 per cage, with 12:12 hr light and dark cycle at $25^{\circ}\pm 2^{\circ}\text{C}$. The animals were maintained on their respective diets and water ad libitum. Animal Ethical Committee's clearance was obtained for the study. Animals were divided into following 4 groups of 6 animals each:

Group I (Control): Standard chow diet

Group II : High Fat Diet

Group III : High fat diet + Methanolic extract of *Ipomoea digitata* (300mg/kg B.wt)

Group IV : High fat diet + standard drug (atorvastatin 1.2 mg/kg B.wt)

Animal diet

The compositions of the two diets were as follows [13]:

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%.

Rats of group III were orally fed with the methanolic extract of *Ipomoea digitata* (300mg/kg body weight) and rats of group IV were fed with standard drug atorvastatin (1.2 mg/kg body weight). Both the methanolic extract of *Ipomoea digitata* and atorvastatin were suspended in 2% tween 80 separately and fed to the respective rats by oral intubation. At the end of 9 weeks all the animals were sacrificed by cervical decapitation after overnight fasting. 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 estimation

Plasma samples were analyzed for total cholesterol, HDL-cholesterol and triglycerides were estimated using Boehringer Mannheim kits by Erba Smart Lab analyzer USA. LDL-cholesterol and VLDL-cholesterol were calculated by using Friedwald method [14]. Ester cholesterol and free cholesterol [15] 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 [16]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides [17], and phospholipids [18]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to assess the atherogenic risk.

Statistical analysis

Results were expressed as mean ± SE of 6 rats in each group. One way analysis of variance (ANOVA) test was used to determine the statistical significance. Significance level was fixed at 0.05.

RESULTS & DISCUSSION

Table 1 illustrates the average body weight in control and experimental animals in each group. The body weight of group II animals were increased significantly (p<0.001) in comparison with normal control group I animals. The average body weight was reduced significantly (p<0.001) by the administration of methanolic extract of Ipomoea digitata (300mg/kg body weight) as well as atorvastatin 1.2mg/kg in comparison with HFD rats (group II).

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

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	126.65±0.82 b ^{NS}	180.66±0.49 b ^{**}	54.01±0.94 b ^{**}
Group II	131.73±0.66 a ^{NS}	276.04±0.68 a ^{**}	144.31±0.98 a ^{**}
Group III	156.40±0.31 a ^{NS, b^{NS}}	232.37±0.92 a ^{NS, b^{**}}	76.29±1.17 a ^{NS, b^{**}}
Group IV	189.67±0.19 a ^{NS, b^{NS}}	255.44±0.60 a ^{NS, b^{**}}	65.76±0.58 a ^{NS, b^{**}}

Values are mean ± SE of 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.

Effect of methanolic extract of Ipomoea digitata on plasma lipid profiles are summarized in Table 2. There was a significant increased level of plasma lipid profile in the group II animals fed with high fat diet in comparison with the normal untreated control animals (group I). Earlier studies reveal significant elevation of lipid parameters in plasma and tissue response to atherogenic diet or high fat diet [19-24]. Treatment of methanolic extract of Ipomoea digitata at the dose of 300mg/kg body weight to rat fed with HFD significantly decreased in the concentration of total cholesterol as compared to HFD rats (group II). However, the administration of methanolic extract of Ipomoea digitata treated rats with HFD showed that the plasma cholesterol was restored to near normal as that of atorvastatin (group IV).

Effect of free and ester cholesterol in plasma and tissue were depicted in table 2,4,5. Significant ($P < 0.001$) increase in levels of both free and ester cholesterol were also observed in plasma of rats fed with high fat diet (group II). This high cholesterol concentration in circulation may be 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 Ipomoea digitata (group III).

Table 2&6 represents the effect of the methanolic extract of Ipomoea digitata on plasma and tissue triglyceride. The concentration of plasma and tissue triglyceride was elevated in rats fed high fat diet (group II) as compared to control rats (group I). HFD rats significant increase in the level of plasma triglyceride due to decrease in the activity of lipoprotein lipase [26,27]. Both plasma and tissue triglyceride levels were significantly reduced in rats treated with methanolic extract of Ipomoea digitata (300mg/kg) and as well as standard drug atorvastatin along with high fat diet in comparison with HFD rats (group II). The plant extract 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 [28].

Table 2- Effect of methanolic extract of Ipomoea digitata on plasma lipid profile in control and experimental rats in each group

[Values are mean \pm SE of 6 rats]

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	111.10 \pm 0.27 b*	26.58 \pm 0.41 b*	84.51 \pm 0.21 b*	97.55 \pm 1.17 b*	90.52 \pm 0.32 b*	2.65 \pm 0.03 b*
Group II	177.97 \pm 0.65 a*	49.80 \pm 0.67 a*	128.16 \pm 0.13 a*	116.03 \pm 3.06 a*	147.08 \pm 0.47 a*	6.21 \pm 0.08 a*
Group III	100.70 \pm 0.23 a*, b*	21.69 \pm 0.33 a*, b*	79.06 \pm 0.5 a*, b*	100.73 \pm 1.35 b*	87.15 \pm 0.68 a*, b*	2.62 \pm 0.03 a*, b*
Group IV	98.03 \pm 0.33 a*, b*	22.18 \pm 0.21 a*, b*	75.85 \pm 0.1 b*	98.58 \pm 1.17 a*, b*	74.25 \pm 0.41 a*, b*	2.16 \pm 0.01 b*

P values : * < 0.001 , ** < 0.05

NS: Non Significant

a \rightarrow group I compared with groups II, III, IV.

b \rightarrow group II compared with groups III, IV.

Details of group I-IV are same as in Table 1.

Effects of methanolic extract of Ipomoea digitata on plasma and tissue phospholipids are presented in table-2&7. The concentration of plasma phospholipids was significantly increased in rats fed HFD (group II) as compared to control animals (group I). This may be due to decreased phospholipase activity [29, 30]. After administration of methanolic extract of

Ipomoea digitata (300mg/kg body weight) along with HFD were shown significantly reduced level of phospholipids in comparison with HFD fed rats (group II).

Table 3 summarized the levels of HDL-cholesterol in plasma of control and experimental rats in each group. The HDL-cholesterol levels were reduced in HFD rats (Group II) as compared to control rats (group I). Treatment of methanolic extract of *Ipomoea digitata* dose at 300mg/kg produced a significant and steady increase in the beneficial HDL-cholesterol concentration in rats fed with high fat diet. It is well known that increased HDL cholesterol levels have a protective role in coronary artery disease [31].

Table 3- Effect of methanolic extract of *Ipomoea digitata* on plasma lipoprotein in control and experimental rats in each group

[Values are mean ± SE of 6 rats]

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Group I	41.95±0.38 b*	74.17±0.29 b*	18.09±0.06 b*
Group II	28.69±0.37 a*	143.10±0.55 a*	29.41±0.09 a*
Group III	38.38±0.51 a*, b*	84.94±0.26 a*, b*	17.42±0.13 a*, b*
Group IV	45.34±0.27 a*, b*	75.59±0.31 a*, b*	14.84±0.08 a*, b*

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.

Effect of methanolic extract of *Ipomoea digitata* on plasma LDL & VLDL- cholesterol levels are presented in table-3. HFD fed rats (group II) are elevated levels of LDL and VLDL-cholesterol when compared with the control rats (group I). High levels of LDL and VLDL-cholesterol are major risk factor for coronary heart disease [32]. Administration of methanolic extract of *Ipomoea digitata* were significantly reduced the levels of LDL and VLDL-cholesterol in plasma when compared with HFD rats (group II).

Table 4- Effect of methanolic extract of *Ipomoea digitata* on tissues ester cholesterol profile in control and experimental rats in each group

[Values are mean ± SE of 6 rats]

Groups	Ester cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	2.56 ± 0.12b*	1.88± 0.02 b*	1.91±0.02 b*
Group II	5.67±0.07a*	3.51±0.10 a*	5.65±0.04 a*
Group III	3.61±0.04 a*	2.30±0.05 a*, b**	3.55±0.06 a*, b**
Group IV	2.73±0.06 a*, b*	1.74±0.07 a*, b*	2.87±0.03 a**

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 5- Effect of methanolic extract of Ipomoea digitata on tissues free cholesterol profile in control and experimental rats in each group

[Values are mean ± SE of 6 rats]

Groups	Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	0.90 ± 0.02b*	0.69± 0.03 b*	10.32±9.90 b*
Group II	1.51±0.03 a*	1.32±0.08 a*	1.67±0.03 a*
Group III	0.93±0.01 a ,b*	0.90±0.02 a **,b*	1.11±0.02 a ,b*
Group IV	0.71 ±0.04 a**	0.84±0.01 a ,b*	0.74±0.01 a ,b**

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 6- Effect of methanolic extract of Ipomoea digitata on tissues Triglyceride level in control and experimental rats in each group

[Values are mean ± SE of 6 rats]

Groups	Triglyceride (mg/g tissue)		
	Liver	Heart	Aorta
Group I	12.40 ± 0.08 b*	13.69 ± 0.07 b*	11.70 ± 0.07 b*
Group II	29.47 ± 0.09 a*	32.31 ± 0.09 a*	25.19 ± 0.06 a*
Group III	20.70 ± 0.07 a **, b*	26.32 ± 0.15 a **, b**	19.28 ± 0.04 a **, b*
Group IV	15.25 ± 0.06 a *, b*	18.35± 0.08 a *, b*	14.46 ± 0.14 a **, b*

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 7- Effect of methanolic extract of Ipomoea digitata on tissues Phospholipids level in control and experimental rats in each group

[Values are mean ± SE of 6 rats]

Groups	Phospholipids (mg/g tissue)		
	Liver	Heart	Aorta
Group I	19.40 ± 0.15 b*	23.48 ± 0.07 b*	9.32 ± 0.05 b*
Group II	29.63 ± 0.09 a*	37.41 ± 0.12 a*	16.31 ± 0.09 a*
Group III	23.63 ± 0.11 a *, b*	32.27 ± 0.07 a *, b*	12.51 ± 0.13 a *, b*
Group IV	19.21 ± 0.06 a **, b*	26.32± 0.09 a *, b*	10.35 ± 0.11 a *, b*

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.

The Atherogenic Index (AI) of the animals fed on HFD was increased many fold in group II animals in comparison with normal group I animals ($P < 0.001$). Administration of methanolic extract of *Ipomoea digitata* was found significantly reduced the atherogenic index when compared to HFD fed rats (group II).

CONCLUSION

From these result it can be concluded that methanolic extract of tuberous root of *Ipomoea digitata* which decreases plasma and tissue lipid profile and lowers the risk of atherosclerosis in high fat diet fed rats.

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