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Hypolipidemic Effect of Various Extracts of Whole Plant of *Mucuna pruriens* (Linn) in Rat Fed with High Fat Diet

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Abstract: The effect of various extracts of whole plant of *Mucuna pruriens* (Family: Fabaceae) was assessed for the hypolipidemic activity in rats, fed high fat diet. High fat diet group of rats showed significant ($p<0.001$) elevation in plasma total and LDL-cholesterol, triglycerides and phospholipids. Administration of methanolic extracts of *Mucuna pruriens* at the dose of 200mg/kg b.wt/day along with high fat diet significantly ($p<0.001$) prevented the rise in the plasma total and LDL-cholesterol, triglycerides and phospholipids than that of other extracts. The ratio of plasma HDL-cholesterol/ total cholesterol and LDL-C/HDL-C ratio were similar in methanolic extract of *Mucuna pruriens* treated group of rats as compared to high fat diet fed rats. Therefore, it was concluded that the methanolic extract of whole plant of *Mucuna pruriens* has definite cardio protective effect against hyperlipidemia.

Key words: *Mucuna pruriens* · Hypolipidemic effect · High fat diet · Rats

INTRODUCTION

Coronary heart disease resulting from progressive atherosclerosis remains the most common cause of morbidity and mortality all over the world [1]. In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of cardiovascular epidemics [2, 3]. The circulatory system disorders are going to be the greatest killer in India by the end of the year 2015 [4]. Hyperlipidemia (mainly increased level of cholesterol or low density lipoprotein (LDL)-cholesterol) is an important risk factor in the initiation and progression of atherosclerotic lesions [5, 6]. The beneficial effect of lowering elevated serum cholesterol level in the prevention of coronary heart disease is well established [7, 8]. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol established [9]. 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 [10]. 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 the use of natural agents due to their cost effectiveness and minimal side-effects [11]. 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 [12].

Mucuna pruriens Linn belongs to the family fabaceae, commonly known as cowhage plant or kapikacho or kevach in Hindi, is the most popular drug in Ayurvedic system of medicine [13]. Traditionally, in India, the seeds of *Mucuna pruriens* are used as a tonic and aphrodisiac for male virility. It has been reported to be antidiabetic [14]. Its different preparations (from seeds) are used for the management of several free radical-mediated diseases such as ageing, rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders. It is also used as an aphrodisiac and in the management of Parkinsonism, as it is a good source of L-dopa [15]. The anti-epileptic and anti-neoplastic activity of methanol extract of *Mucuna pruriens* has been reported [16]. It had been reported analgesic and anti-inflammatory [17]. It is also used as a fertility agent in men [18].

Literature survey revealed that there is a lack of enough scientific reports regarding hypolipidemic activity of the whole plant of *Mucuna* pruriens (Linn.). Hence the objective of the present research work was to investigate the hypolipidemic activity of various extracts of the whole plant of *Mucuna pruriens* (Linn) in rat fed with high fat diet.

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MATERIALS AND METHODS

Collection and Identification of Plant Materials: The whole plant of *Mucuna pruriens* (Linn), were collected from Neiyur dam, Kanyakumari 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 *Mucuna pruriens* (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 petroleum ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus [19] 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 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 [20].

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°±2°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 6 groups of 6 animals each:

Group I (Control)	:	Standard chow diet.
Group II	:	High Fat Diet.
Group III	:	High fat diet + Pet.ether extract of *Mucuna pruriens* (200mg/kg body weight).
Group IV	:	High fat diet + Ethyl acetate extract of *Mucuna pruriens* (200mg/kg body weight).
Group V	:	High fat diet + Methanol extract of *Mucuna pruriens* (200mg/kg body weight).
Group VI	:	High fat diet + standard drug atorvastatin (1.2 mg/kg body weight)

Animal Diet: The compositions of the two diets were as follows [21]:

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 groups III, IV and V were orally fed with the various extracts of *Mucuna pruriens* (pet.ether, ethyl acetate and methanol) and rats of group VI were fed with standard drug atorvastatin. Both the *Mucuna pruriens* extracts 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. Blood was collected in heparinized 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 Estimation: 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 [22]. Ester cholesterol [23] and free cholesterol [23] 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.* [24]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides [25] and phospholipids [26]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to access 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 AND DISCUSSION

Table 1 shows the average body weight in control and experimental animals in each group. The average body weight gain of rats in all the six groups was increased after 9 weeks of experimental period. But high

fat diet fed rats (group II) had a significant increase in body weight compared with other group rats. After administration of various extracts of *Mucuna pruriens* (petroleum ether, ethyl acetate and methanolic extract) it was found to be decreased in body weight. But the administration of the methanolic extract of *Mucuna pruriens* were found to more significantly decreased ($p<0.001$) the body weight gain when compared to high fat diet rats group (II).

Table 2 shows the effect of various extracts of *Mucuna pruriens* on plasma lipid profile in control and experimental rats in each group. Total cholesterol levels were increased in high fat diet fed rats (group II) as compared to control rats (group I). Results show that treatment with high fat diet significantly increased the concentration of plasma and tissue lipids as reported earlier revealing that significant elevation of plasma and tissue lipid parameters in response to atherogenic diet and cholesterol feeding [27-32]. Administration of ethyl acetate and methanolic extract of *Mucuna pruriens* (dose 200mg/kg body weight) to rat fed with HFD significantly decreased in the concentration of total cholesterol as compared to HFD rats (group II). But the administration of methanolic extract of *Mucuna pruriens* treated rats with HFD showed that the plasma cholesterol was restored to near normal as that of atorvastatin (group VI).

Effect of free and ester cholesterol in plasma and tissue were present in Tables 2, 4&5. 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). 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 [33]. Increased intake of saturated fatty acids results an increased cholesterol production in liver [34]. Both plasma free and ester cholesterol reduced remarkably on treating the HFD rats with methanolic extract of *Mucuna pruriens*. This lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of fecal sterol [32].

Effect of the various extracts of *Mucuna pruriens* on plasma and tissue triglyceride are presented in Tables 2&6. 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 [35, 36]. Both plasma and tissue triglyceride levels were significantly reduced in rats treated with ethyl acetate and methanolic extracts of *Mucuna pruriens* (200mg/kg b.wt) 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 *Mucuna pruriens* significantly reduced the triglyceride when compared with other two extracts. 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 [37].

Effect of various extracts of *Mucuna pruriens* on plasma and tissue phospholipids are presented in Tables 2&7. 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 [38, 39]. After treatment of methanolic extract of *Mucuna pruriens* (doses 200mg/kg body weight) along with HFD phospholipids levels were significantly reduced as compared to HFD fed rats (group II). The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases [21].

Table 3 shows the levels of HDL cholesterol in plasma of control and experimental rats in each group. The HDL cholesterol levels increased in high fat diet rats (Group II) as compared to control rats (group I). But the administration of methanolic extract of *Mucuna pruriens* was found significantly elevated the HDL-cholesterol levels when compared with other extracts. Several studies show that an increase in HDL –cholesterol is associated with a decrease in coronary risk [40].

Effect of various extracts of *Mucuna pruriens* on plasma LDL & VLDL levels are presented in Table 3. HFD fed rats (group II) are elevated levels of LDL and VLDL-cholesterol when compared with the control (group I). High levels of LDL and VLDL- cholesterol are major risk factor for coronary heart disease [41]. Studies show that both LDL and VLDL have a positive role in atherogenesis [42]. Administration of methanolic extract of *Mucuna pruriens* markedly reduced the level of LDL, VLDL-cholesterol in plasma when compared with other extracts. Reduced levels of LDL & VLDL in methanolic extract of *Mucuna pruriens* on HFD fed rats may be possibly due to increase with catabolism of LDL.

The ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol are presented in Table 3. High fat diet rats caused significant ($P<0.001$) increase in the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol.

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	124.77±0.21^{bNS}	177.50±6.68 ^{b**}	52.88±2.76 ^{b**}
Group II	123.83±0.79^{aNS}	288.33± 10.77 ^{a**}	162.83 ± 3.91 ^{a**}
Group III	152.33 ± 0.95$^{aNS, bNS}$	262.50± 10.78$^{aNS, b*}$	119.33 ± 5.93 $^{aNS, b*}$
Group IV	152.33±0.95^{aNS,bNS}	269.17±7.35$^{aNS, b*}$	111.83 ± 3.05 $^{aNS, b*}$
Group V	152.33± 0.95^{aNS,bNS}	222.50 ± 8.34$^{aNS, b**}$	66.83 ± 5.74 $^{aNS, b*}$
Group VI	195±9.74^{aNS,bNS}	259.17 ± 10.44^{aNS,b**}	64.5 ± 11.58 $^{aNS, b*}$

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, V, VI.

b → Group II compared with groups III, IV, V, VI.

Group I : Standard chow diet.(Control)

Group II : High Fat Diet.

Group III : High fat diet + Pet.ether extract of *Mucuna pruriens* (200mg/kg b.wt)

Group IV : High fat diet + Ethyl acetate extract of *Mucuna pruriens* (200mg/kg b.wt)

Group V : High fat diet + Methanol extract of *Mucuna pruriens* (200mg/kg b.wt)

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

Table 2: Effect of various extracts of *Mucuna pruriens* on plasma lipid profile in control and experimental rats in each group

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	112.94±1.27b*	25.04 ±0.91 b*	87.39 ±0.61 b*	101.82 ±0.34 b*	80.62 ±1.11 b*	1.89 ±0.03 b*
Group II	171.67±1.74a*	43.40 ±0.78 a*	128.26 ± 1.47 a*	145.48 ±0.42 a*	150.12 ± 1.17 a*	4.18 ±0.17 a*
Group III	153.30 ±3.87 a**, b*	39.74 ±0.97 a*, b*	120.17 ± 2.25 a*, b**	141.79 ± 0.51 a*, b*	140..43 ± 1.37 a*, b*	2.76 ± 0.04 a**, b*
Group IV	113.76 ± 0.79 a*, b*	25.44 ±0.58 a*, b*	88.33 ±1.02 a*, b*	125.58 ±0.46 a*, b*	115.21 ± 0.52 a*, b**	2.21 ±0.03 a*, b*
Group V	99.63 ±0.51 a*, b*	22.35 ±0.45 a*, b*	77.28 ±0.72 a*, b*	108.07 ±0.42 a*, b*	74.41 ±0.96 a*, b*	1.72 ±0.02 a*, b*
Group VI	97.22 ±0.95 a*, b*	22.46 ±0.55 a*, b*	74.76 ± 1.066 a*, b*	100.46 ±0.29 a*, b*	65.20 ±0.90 a*, b*	1.69 ±0.01 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, V, VI.

b → Group II compared with groups III, IV, V, VI.

Details of group I-VI are same as in Table 1

Table 3: Effect of various extracts of *Mucuna pruriens* on plasma lipoprotein in control and experimental rats in each group

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	LDL- c/HDL-c ratio	HDL-c/ TC ratio
Group I	59.41 ±0.88 b*	36.61 ± 1.03 b*	16.16 ± 0.22 b*	0.61 ± 0.03 b*	0.52 ± 0.007 b*
Group II	41.41 ± 1.97 a*	100.22 ± 1.63 a*	30.03 ± 0.23 a*	2.45 ± 0.14 a*	0.23 ± 0.009 a*
Group III	42.73 ± 0.99 a*, b*	54.82 ± 1.22 a*, b**	27.08 ± 1.32 a*, b*	1.25 ± 0.04 a*, b*	0.36 ± 0.005 a*, b**
Group IV	51.58 ± 0.60 a*, b*	39.15 ± 1.00 a**, b*	23.04 ± 0.10 a*, b*	0.76 ± 0.03 a*, b*	0.45 ± 0.006 a**, b*
Group V	57.83 ± 0.71 a*, b*	26.78 ± 1.13 a*, b*	14.88 ± 0.19^{a*},b*	0.46 ± 0.02 a*, b*	0.57 ± 0.009 a*, b*
Group VI	57.28 ± 0.31 a*, b*	26.93 ± 0.73 a*, b*	13.09 ± 0.20 a*, b*	0.46 ± 0.01 a*, b*	0.58 ± 0.004 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, V, VI.

b→ Group II compared with groups III, IV, V, VI.

Details of group I-VI are same as in Table 1

Table 4: Effect of various extracts of *Mucuna pruriens* on tissues ester cholesterol profile in control and experimental rats in each group

	Ester cholesterol (mg/g tissue)		
Groups	Liver	Heart	Aorta
Group I	1.94 ± 0.05^{b*}	2.73± 0.09^{b*}	2.02±0.42^{b*}
Group II	3.25±0.13^{a*}	7.07±0.16^{a*}	6.81±0.23^{a*}
Group III	2.88±0.09^{a*,b*}	4.97±0.12^{a*,b**}	6.34±0.15^{a*,b**}
Group IV	2.58±0.06^{a*,b*}	4.10±0.09^{a*,b*}	4.98±0.24^{a*,b*}
Group V	1.90±0.07^{a*,b*}	2.95±0.03^{a*,b*}	2.69±0.09^{a*,b*}
Group VI	1.98±0.09^{a*,b*}	2.94±0.08^{a*,b*}	2.83±0.11^{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, V, VI.

b → Group II compared with groups III, IV, V, VI.

Details of group I-VI are same as in Table 1

Table 5: Effect of various extracts of *Mucuna pruriens* on tissues free cholesterol profile in control and experimental rats in each group

	Free cholesterol (mg/g tissue)		
Groups	Liver	Heart	Aorta
Group I	0.84 ± 0.06^{b*}	0.73± 0.02^{b*}	0.49±0.03^{b*}
Group II	1.31±0.04^{a*}	1.06±0.04^{a*}	2.43±0.17^{a*}
Group III	1.26±0.05^{a**,b*}	1.00±0.04^{a*,b*}	1.74±0.08^{a*,b**}
Group IV	1.02 ±0.03^{a*,b*}	0.81±0.03^{a*,b**}	1.06±0.05^{a*,b*}
Group V	0.85±0.02^{a*,b*}	0.63±0.03^{a*,b*}	0.70±0.05^{a*,b*}
Group VI	0.86±0.04^{a*,b*}	0.64±0.04^{a*,b*}	0.63±0.04^{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, V, VI.

b → Group II compared with groups III, IV, V, VI.

Details of group I-VI are same as in Table 1

Table 6: Effect of various extracts of *Mucuna pruriens* on tissues Triglyceride level in control and experimental rats in each group

	Triglyceride (mg/g tissue)		
Groups	Liver	Heart	Aorta
Group I	8.31 ± 0.10 b*	10.78 ± 0.11 b*	10.08 ± 0.17 b*
Group II	28.56 ± 0.16 a*	48.24 ± 0.17 a*	22.14 ± 0.19 a*
Group III	27.75 ± 0.13 a**, b*	42.87 ± 0.10 a*, b*	21.09 ± 0.16 a*, b**
Group IV	20.99 ± 0.16 a*, b*	37.84 ± 0.12 a**, b*	17.76 ± 0.09 a*, b*
Group V	10.65 ± 0.09 a*, b*	18.78± 0.16 a*, b*	13.15 ± 0.09 a*, b**
Group VI	12.29 ± 0.10 a*, b*	21.48 ± 0.12 a**, b*	13.22 ± 0.12 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, V, VI.

b → Group II compared with groups III, IV, V, VI.

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

Table 7: Effect of various extracts of *Mucuna pruriens* on tissues Phospholipids level in control and experimental rats in each group

	Phospholipids (mg/g tissue)		
Groups	Liver	Heart	Aorta
Group I	17.52 ± 0.23 b*	23.50 ± 0.27 b*	8.81 ± 0.10 b*
Group II	25.81 ± 0.24 a*	36.06 ± 0.29 a*	16.64 ± 0.09 a*
Group III	25.85 ± 0.05 a**, b*	34.19 ± 0.15 a*, b*	15.24 ± 0.12 a*, b*
Group IV	24.61 ± 0.32 a*, b**	30.31± 0.36 a*, b*	13.25 ± 0.10 a*, b*
Group V	18.72 ± 0.17 a*, b*	25.39 ± 0.18 a*, b**	10.67 ± 0.10 a*, b*
Group VI	20.37 ± 0.16 a**, b*	27.17 ± 0.23 a*, b*	11.11 ± 0.12 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, V, VI.

b → Group II compared with groups III, IV, V, VI.

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

These results are consistent with earlier reports [29, 30]. Administration of ethyl acetate and methanolic extract of *Mucuna pruriens* along with HFD was found significantly reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol when compared to HFD group (II). But the methanolic extract of *Mucuna pruriens* along with HFD (group V) was showed similar result to standard group rats (VI).

Atherogenic index is used as a marker to assess the susceptility of atherogenesis. It was markedly increased on feeding HFD to rats. The methanolic extract of *Mucuna pruriens* significantly decreased atherogenic index when compared with other two extracts. But, when the methanolic extract of *Mucuna pruriens* was compared with standard group of rats were found to have similar result.

In conclusion, from these results it can be concluded that methanolic extract of whole plant of *Mucuna pruriens* contains active components which decreases plasma lipid profile and lowers the risk of atherosclerosis in high fat diet.

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