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Lipid Lowering Effect of Various Extracts of Whole Plant of *Borreria hispida* (Linn) in Rat Fed with High Fat Diet

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The objective of the present investigation is to evaluate the lipid lowering effect of various extract of whole plant of *Borreria hispida* (Linn) in high fat fed rats. A total number of 36 animals were divided into 6 groups of six each. Group I normal, group II high fat diet, group III-high fat diet plus pet. ether extract (200 mg/kg b.wt), group IV high fat diet plus ethyl acetate extract (200 mg/kg b.wt), group V high fat diet plus methanol extract (200 mg/kg b.wt), group VI high fat diet plus standard drug atorvastatin (1.2 mg/kg b.wt). Administration of high fat diet caused a significant rise ($p < 0.001$) in the plasma and tissues levels of total cholesterol, triglycerides, phospholipids, LDL-cholesterol, VLDL-cholesterol and total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol ratios. Simultaneous administration of various extract of *Borreria hispida* significantly reduces the plasma and tissues lipid and lipoprotein levels. The methanolic extract of *Borreria hispida* was found significantly decreased the levels of lipid and lipoprotein in plasma and tissues (liver, heart, aorta) than that of other extracts. It is concluded that the methanolic extract of *Borreria hispida* has potent lipid lowering effects in high fat fed rats.

Key Words: *Borreria hipida*, Hypolipidemic effect, High fat diet, Rats.

INTRODUCTION

Hyperlipidemia is a highly predictive risk for atherosclerosis coronary artery disease and cerebral vascular disease[1]. Atherosclerosis of arteries is a generalized disease of the arterial network known as progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plaques in the walls of large and or medium seized coronary arteries and which reduces blood flow to the myocardium-called coronary artery disease[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].

Borreria hispida belongs to the family Rubiaceae. It is widely distributed throughout India, up to 900 m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-α gene expression, antioxidant redox status, protein metabolism in STZ diabetic rats. Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor (Vasanthi HR, 2009)[7]. Present work devoted to evaluate the lipid lowering effect of various extracts of the whole plant of *Borreria hispida* (Linn) on rat fed with high fat diet.

EXPERIMENTAL

Collection and identification of plant materials: The whole plant of *Borreria hispida* (Linn), were collected from Naserath, Tuticorin 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 *Borreria hispida* (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 ºC) by hot continuous percolation method in Soxhlet apparatus[8] for 24 h. Then the marc was dried and then subjected to ethyl acetate (76-78 ºC) for 24 h, then marc was dried and then it was subjected to methanol for 24 h. 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[9].

Animals and treatment: Male Wister rats of 16-19 weeks age, weighing 150-175 g 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 h light and dark cycle at 25 ± 2 ºC. 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 *Borreria hispida* (200 mg/kg body weight). Group IV : High fat diet + ethyl acetate extract of *Borreria hispida* (200 mg/kg body weight). Group V : High fat diet + methanol extract of *Borreria hispida* (200 mg/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[10]:

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 and 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 and choline mixture 0.5 %, cholesterol 0.4 %.

Rats of groups III, IV and V were orally fed with the various extracts of *Borreria hispida* (pet. ether, ethyl acetate and methanol) and rats of group VI were fed with standard drug atorvastatin. Both the *Borreria hispida* 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: The 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[11]. Ester cholesterol[12] and free cholesterol[12] 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*.[13]. Extracts were used for the estimation of ester cholesterol and free cholesterol, triglycerides[14] and phospholipids[15]. 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

The average body weight was increased in high fat diet fed rats compared to control groups. After administration of various extracts of *Borreria hispida* (petroleum ether, ethyl acetate and methanolic extract) it was found to be decreased in body weight (Table-1). But the administration of the methanolic extract of *Borreria hispida* were found to more significantly decreased ($p < 0.001$) the body weight gain when compare to high fat diet rats group (II).

Effect of various extracts of *Borreria hispida* on plasma lipid profiles are shown in Table-2. Results clearly show that in high fat diet fed rats, there were found increased plasma and tissues lipids levels. Earlier studies reveal significant elevation of lipid parameters in plasma and tissue response to atherogenic diet or high fat diet[16-21]. Administration of ethyl acetate and methanolic extract of *Borreria hispida* (dose 200 mg/kg body weight) to rat fed with high fat diet significantly decreased in the concentration of total cholesterol as compared to high fat diet rats (group II). But the administration of methanolic extract of *Borreria hispida* treated rats with high fat diet 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 and 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[22]. Increased intake of saturated fatty acids results an increased cholesterol production in liver[23]. Both plasma free and ester cholesterol reduced remarkably on treating the high fat diet rats with methanolic extract of *Borreria hispida*. This

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	125.77 ± 0.25^{bNS}	$177.50 \pm 6.68^{b**}$	$51.73 \pm 2.76^{b**}$
Group II	124.16 ± 0.87^{aNS}	$288.33 \pm 10.77^{a**}$	$164.17 \pm 3.91^{a**}$
Group III	$150.33 \pm 0.61^{aNS,bNS}$	$262.50 \pm 10.78^{aNS,b*}$	$112.17 \pm 5.93^{aNS,b*}$
Group IV	$131.33 \pm 3.95^{aNS,bNS}$	$249.17 \pm 7.35^{aNS,b*}$	$117.84 \pm 3.05^{aNS,b*}$
Group V	$174.66 \pm 0.61^{aNS,bNS}$	$242.50 \pm 8.34^{aNS,b**}$	$67.84 \pm 5.74^{aNS,b*}$
Group VI	$195 \pm 9.74^{aNS,bNS}$	$259.17 \pm 10.44^{aNS,b**}$	$64.5 \pm 11.58^{aNS,b*}$

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, 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 *Borreria hispida* (200 mg/kg b.wt). Group IV: High fat diet + ethyl acetate extract of *Borreria hispida* (200 mg/kg b.wt). Group V: High fat diet + methanol extract of *Borreria hispida* (200 mg/kg b.wt). Group VI: High fat diet + standard drug atorvastatin (1.2 mg/kg b.wt).

TABLE-2
EFFECT OF VARIOUS EXTRACTS OF *Borreria hispida* ON PLASMA LIPID PROFILE IN CONTROL AND EXPERIMENTAL RATS IN EACH GROUP [VALUES ARE MEAN ± SE OF 6 RATS]

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	$111.98 \pm 1.02^{b*}$	$25.04 \pm 0.91^{b*}$	$86.94 \pm 0.92^{b*}$	$103.16 \pm 0.63^{b*}$	$80.06 \pm 0.92^{b*}$	$1.87 \pm 0.03^{b*}$
Group II	$173.61 \pm 1.56^{a*}$	$43.40 \pm 0.78^{a*}$	$130.21 \pm 2.13^{a*}$	$144.13 \pm 0.87^{a*}$	$151.74 \pm 1.26^{a*}$	$4.18 \pm 0.21^{a*}$
Group III	$130.12 \pm 1.19^{a**,b**}$	$39.74 \pm 0.97^{a**,b**}$	$100.32 \pm 1.02^{a**,b*}$	$142.47 \pm 0.48^{a*,b*}$	$135.52 \pm 1.08^{a**,b*}$	$2.76 \pm 0.03^{a*,b*}$
Group IV	$115.64 \pm 1.07^{a**,b*}$	$25.44 \pm 0.58^{a*,b**}$	$90.2 \pm 1.38^{a*,b**}$	$126.16 \pm 0.63^{a**,b*}$	$116.10 \pm 1.67^{a*,b*}$	$2.25 \pm 0.03^{a*,b*}$
Group V	$101.42 \pm 0.89^{a*,b*}$	$22.35 \pm 0.45^{a*,b*}$	$79.03 \pm 1.06^{a*,b*}$	$109.81 \pm 0.25^{a*,b*}$	$74.01 \pm 0.86^{a*,b*}$	$1.75 \pm 0.03^{a*,b*}$
Group VI	$97 \pm 0.63^{a*,b*}$	$22.46 \pm 0.55^{a*,b*}$	$74.53 \pm 0.02^{a*,b*}$	$100.36 \pm 0.37^{a*,b*}$	$65.78 \pm 0.74^{a*,b*}$	$1.71 \pm 0.02^{a*,b*}$

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.

lipid lowering effect may be due to the inhibition of hepatic cholesterogenesis or due to the increase in excretion of faecal sterol[9].

Effect of the various extracts of *Borreria hispida* on plasma and tissue triglyceride are presented in Tables 2 and 6. The concentration of plasma and tissue triglyceride was elevated in rats fed high fat diet (group II) as compared to control rats (group I). High fat diet rats significant increase in the level of plasma triglyceride due to decrease in the activity of lipoprotein lipase[24,25]. Both plasma and tissue triglyceride levels were significantly reduced in rats treated with ethyl acetate and methanolic extracts of *Borreria hispida* (200 mg/kg b.Wt) and as well as standard drug atorvastatin along with high fat diet when compared with rats fed with high fat diet (group II). Administration of methanolic extract of *Borreria hispida* was 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[26].

Effect of various extracts of *Borreria hispida* on plasma and tissue phospholipids are presented in Tables 2 and 7. The concentration of plasma phospholipids was significantly increased in rats fed high fat diet (group II) as compared to control animals (group I). This may be due to decreased phospolipase activity[27,28]. After treatment of methanolic extract of *Borreria hispida* (doses 200 mg/kg body weight) along with high fat diet phospholipids levels were significantly reduced as compared to high fat diet fed rats (group II). The reduced concentration of phospholipids may also be due to the enhanced activity of phospholipases[9].

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 *Borreria hispida* 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[29].

The LDL and VLDL-cholesterol levels of high fat diet fed rats are shown in Table-3. High fat diet 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[30]. Studies show that both LDL and VLDL have a positive role in atherogenesis[31]. Administration of methanolic extract of *Borreria hispida* was markedly reduced the level of LDL, VLDL-cholesterol in plasma when compared with other extracts. Reduced levels of LDL and VLDL in methanolic extract of *Borreria hispida* on high fat diet fed rats may be possibly due to increase with catabolism of LDL.

Ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol is 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. These results are consistent with earlier reports[18,19]. Administration of ethyl acetate and methanolic extract of *Borreria hispida* along with high fat diet was found significantly reduced the ratios of total cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol when compared to high fat diet group (II). But the methanolic extract of *Borreria hispida* along with high fat diet (group V) was showed similar result to standard group rats (VI).

Atherogenic index is used for determination of the susceptibility of atherogenesis. It was significantly increased on high fat diet fed rats (group II) as compared to control rats (group I).

TABLE-3
EFFECT OF VARIOUS EXTRACTS OF *Borreria hispida* 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)	LDL-c/HDL-c ratio	HDL-c/TC ratio
Group I	$59.69 \pm 0.87^{b*}$	$36.36 \pm 1.29^{b*}$	$16.01 \pm 0.16^{b*}$	$0.60 \pm 0.03^{b*}$	$0.54 \pm 0.01^{b*}$
Group II	$41.93 \pm 1.82^{a*}$	$101.34 \pm 3.02^{a*}$	$30.34 \pm 0.25^{a*}$	$2.45 \pm 0.17^{a*}$	$0.24 \pm 0.01^{a*}$
Group III	$47.32 \pm 0.56^{a**,b*}$	$55.70 \pm 1.35^{a*,b*}$	$27.08 \pm 0.22^{a**,b**}$	$1.18 \pm 0.03^{a*,b*}$	$0.36 \pm 0.01^{a*,b**}$
Group IV	$51.37 \pm 0.64^{a*,b*}$	$41.21 \pm 1.15^{a**,b*}$	$23.05 \pm 0.19^{a*,b**}$	$0.82 \pm 0.01^{a*,b*}$	$0.44 \pm 0.01^{a*,b*}$
Group V	$58.05 \pm 0.62^{a*,b*}$	$28.53 \pm 1.49^{a*,b*}$	$14.79 \pm 0.17^{a*,b*}$	$0.49 \pm 0.03^{a*,b*}$	$0.57 \pm 0.01^{a*,b*}$
Group VI	$56.63 \pm 0.55^{a*,b*}$	$27.21 \pm 1.17^{a*,b*}$	$13.15 \pm 0.14^{a*,b*}$	$0.48 \pm 0.02^{a*,b*}$	$0.58 \pm 0.01^{a*,b*}$

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 *Borreria hispida* 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	1.82 ± 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*}

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 *Borreria hispida* 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	0.81 ± 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*}

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 *Borreria hispida* 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	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*}

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 *Borreria hispida* 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	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*}

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.

Atherogenic index significantly decreased on treatment with methanolic extract of *Borreria hispida* on high fat fed rats.

Conclusion

In this study, it was found that methanolic extract of whole plant of *Borreria hispida* have good hypolipidemic activity on high fat diet rats. Further, investigations are required to gain more insight in to the possible mechanism of action.

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