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Lipid Lowering potential of *Andrographis paniculata* (Nees)

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Abstract

Aim: Atherosclerosis and associated complications is now the major cause of myocardial morbidity and mortality worldwide. Therefore we have selected the Andrographis paniculata for the development of lipid lowering drug. **Material and Methods:** The lipid lowering activity of mixture of andrographaloides isolated from the leaves of the *Andrographis paniculata* has been studied in Triton and cholesterol fed hyperlipidemic rats (in vivo). **Results:** Serum lipids were found to be lowered by andrographaloides (at 50 mg/kg.) in Triton WR-1339 induced hyperlipidemia in experimental animals. Chronic feeding of this mixture of andrographolides (at 25 mg/kg) in animals, simultaneously fed with high fat diet (HFD) for 30 days caused lowering in the lipid and apoprotein levels of very low density (VLDL) and low density lipoproteins (LDL) It has also increased high density lipoprotein (HDL). Andrographaloides activated lipolytic enzymes in plasma and liver lipids. The hypolipidemic activity of the andrographaloides mixture is mediated through increased faecal bile acid excretion and enhanced plasma lecithin-cholesterol acyl transferase activity. **Conclusion:** Mixture of Andrographolides was found to lower the lipids in experimental animals.

Keywords: *Andrographis panniculata*, Andrographaloide, Lipid lowering activity, Triton , HFD models.

Introduction

Atherosclerosis and associated complications is now the major cause of myocardial morbidity and mortality worldwide. Elevated level of plasma concentration of cholesterol especially low density lipoprotein (LDL) and triglyceride along with free radicals oxidative stress are recognized as leading cause in the development of atherosclerosis and coronary heart diseases. Several drugs are being used in the treatment of dyslipidemia. Treatment of hyperlipidemia using statins has been used to decrease serum levels of cholesterol and triglyceride. Statin such as atrovastatin, lovastatin, fluvastatin, simvastatin, and pravastatin are HMG-CoA reductase inhibitirs which act by inhibiting cholesterol synthesis and up regulate LDL receptors in liver. However common side effects of statins are myositis, arthralgias, gastrointestinal upset and elevated liver function test. Thus there is a need of the therapeutic benefits of several antidislipidemic drugs while simultaneously reducing the severe side effects.

Andrographis paniculata (Nees) belongs to the Natural Order Acanthaceae. *A. paniculata* is a medicinal plant, commonly known as king of bitters. *A. panniculata* was reported to possess anti-inflammatory[1] anticancer[2,3] immunomodulatory[4]

antiinfective effects[5] antihepatoprotective[6] antiatherosclerotic[7,8] antihyperglycemic[9,10] and antioxidative[11,12] activities. The present study was undertaken to investigate the lipid lowering property of andrographolide isolated from *A .paniculata*.

Materials and Methods

Collection of plant materials

A. paniculata plant grows naturally in tidal forests along the East and West coastal areas up to Maharastra and in Andaman Island. The *A. paniculata* leaves were purchased from Lucknow market and was authenticated by botany division of the Central Drug Research Institute (CDRI), Lucknow.

Extraction/Fractionation and Isolation procedure

The shade dried leaves (1.0 Kg) were powdered and extracted with 95% ethanol (4x2.0 lit). Combined extract was filtered and concentrated under reduced pressure below 50ºC in a rotavapour to a green viscous mass (31.2 g). The ethanol extract thus obtained was macerated with chloroform and concentrated in a rotavapour to get chloroform soluble fraction (4.2 g). The chloroform soluble fraction was dissolved in 25 ml of methanol and left in a refrigerator. White deposit in the solution was filtered and identified as andrographolides mixture by physicochemical methods reported in the literature. This mixture of andrographolides was used for biological screening of lipid lowering activity.

Experimental animals

Male adult rats of Charles foster strain (150 -200 g) bred in the animal house of the Institute were kept in a room with controlled temperature at 25-26 °C, humidity 60 -80% and 12/12 hours light/dark cycle, light from 8.00-20.00 hours under hygienic conditions. The animal had free access to the normal diet and water ad libitum.

Lipid lowering activity in Triton induced Hyperlipidemic rat model

The lipid lowering activity of andrographolide was evaluated in Triton treated hyperlipidemic rats. The rats were divided into control, Triton treated and Triton plus andrographolide treated groups containing six rats in each group. In the acute experiment Triton WR-1339 (Sigma Chemical Company, St. Louis, M O, USA) was administered (400 mg/kg) by intraperitonial injection for 18 hours. Andrographolide and Gemfibrozil (Cipla Ltd. Bombay, India) were macerated with 0.2% aqueous gum acacia suspension and fed orally Simultaneously with Triton in the chronic experiment. Hyperlipidemia was produced by feeding with high fat diet (Novo Nordisk, Denmark) once a day for 30 days. Drugs were administered orally (50 mg/kg.) simultaneously with cholesterol in drug treated groups. Control animals received same amount of normal saline or groundnut oil. At the end of experiments rats were fasted overnight and blood was withdrawn. The animals were killed and the liver was excised immediately.

Plasma lecithin: cholesterol acyl transferase (LACT) activity[13] and Post heparin lipolytic activity (PHLA) were assayed[14]. Serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanimic precipitation methods.[15] Serum as well as lipoproteins were analysed for their total cholesterol (TC), phospholipids (PL), triglyceride (TG) and protein by standard procedures reported earlier.[16,17]

Liver was homogenised (10% w/v) in cold 100 mM phosphate buffer, pH 7.2 and used for the assay of total lipolytic activity of the lipid extract of each homogenate was used for the estimation of TC, PL, TG and protein. The rat faeces were collected from all groups throughout 30 days and processed for the estimation of cholic acid and de-oxy cholic acid.[18] Data were analyzed using student's t-test. Hyperlipidemic groups were compared with control and andrographolide treated hyperlipidemic. $P<0.05$ was considered as significant.

Results

Effect of mixture of andrographolides in Triton induced hyperlipidaemia

The acute administration of Triton WR-1339 caused a marked increase in the serum levels of TC (+ 2.87 F), PL (+2.44 F), TG (+2.87 F) and protein (+2.07 F). Treatment with these extract caused reversal in these levels of TC (-25%), PL (-23%), TG (-23%) and protein (-21%). The lipid lowering activity of andrographolide in the hyperlipidemic rats was comparable to that of Gemfibrozil (Table 1).

Table 1: Lipid lowering activity of andrographolide in triton treated hyperlipemic rats

Experimental Schedule	Total Cholesterol [a] (mg/dl)	Phospholipid [a] (mg/dl)	Triglyceride [a] (mg/dl)	Protein[b] (g/dL)
Contol	88.72±5.37	90.33±8.00	85.40±6.14	7.33±0.26
Triton treated	254.66±20.14*** (+2.87F)	220.44±16.14*** (+2.87F)	245.81±20.23*** (2.80F)	15.18±1.10*** (2.07F)
Triton + andrographolide	188.88±13.68*** (-25)	188.27±*** (-23)	170.37±14.23*** (-23)	11.00±0.78** (-21)
Triton+Gemfibrozil (standard Drug)	1.65±13.00*** (-35)	140.10±10.17*** (-33)	160.20±14.11*** (-35)	11.08±0.66*** (-27)

Values are mean ± SD from 6 rats ***P< 0.001, **P< 0.01. Triton group compared with control, triton and drug treated compared with triton.

Effect of mixture of andrographolides on lipid composition in serum lipoproteins and liver

The data showed that the administration of HDF in rats increased their serum levels of TC, PL and TG by 2.97, 2.86 and 1.88 fold respectively. Feeding with these extract and Gemfibrozil reversed the levels of these serum lipids (26, 25 and 27%) in cholesterol and extract treated animals. The analysis of hyperlipidemic serum showed a marked increase in the levels of lipids and apoproteins constituting β-lipoproteins and these effects were pronounced for VLDL-TG (-23%) and LDL-TC (-22%). Treatment with these extract and Gemfibrozil significantly reduced these levels of VLDL lipids (-28%) as well as LDL-TC (-38%), PL (-33%), TG (-29%) and apo-LDL (-30%) respectively in hyperlipidemic rats. At the same time the decreased levels of HDL-lipids and apo-HDL in these animals were partially recovered. The increased levels of TC, PL, TG and protein (1.73, 1.65, 1.48 and 1.44 F) in liver of cholesterol fed rats were observed to be lowered by their treatment with this compound (Table 2).

Effect on lipolytic enzymes

HDF feeding caused the inhibition of plasma LCAT (-49%) and PHLA (-38%) respectively and total lipolytic activity (-44%) in liver. Treatment with these extract and Gemfibrozil partially reactivated these lipolytic activities in plasma and liver of hyperlipidemic rats.

Effects on faecal excretion of bile acids

Feeding with HDF caused a significant decrease in faecal excretion of cholic acid (-41 %) and deoxycholic acid (-55%) and these levels were shown to be recovered by the treatment with rohitukine (+28% and +54%) and gemfibrozil (+25 and +44%) in HDF and extract fed animals (Table 3).

Table 2: Effect of andrographolide and Gemfibrozil on blood lipids and lipolytic enzymes in hyperlipidemic rats.

Parameters	**Control**	**Cholesterol treated**	**Cholesterol and andrographolide treated**	**Cholesterol and Gemfibrozil treated**
Serum				
Total Cholesterol [a]	86.66±5.48	258.11±20.62*** (+2.97F)	190.44±13.88*** (-26)	170.84±13.69*** (-34)
Phospholipid [a]	83.47±6.00	239.22±19.39*** (+2.86F)	180.27±14.48*** (-25)	166.66±10.82*** (-30)
Trigliyceride [a]	106.88±9.00	201.93±14.44*** (+1.88F)	148.21±5.59*** (-26)	128.37±7.88*** (-36)
Protein [b]	6.38±0.17	12.27±1.00*** (+2.85F)	8.98±0.17*** (-27)	8.00±0.47*** (-35)
VLDL				
Total Cholesterol [a]	8.32±0.40	32.40±2.20*** (+3.89F)	25.00±1.50*** (-23)	21.37±1.62*** (-34)
Phospholipid [a]	15.00±0.48	31.24±2.00*** (2.08F)	27.00±1.64*** (-26)	20.14±2.00*** (-35)
Triglyceride [a]	40.37±2.82	90.87±6.82*** (+2.25F)	72.30±4.00*** (-23)	65.12±5.37*** (-28)
Apoprotein[b]	6.40±0.38	12.64±0.87*** (+1.97F)	9.40±0.38*** (-25)	9.00±0.27*** (-28)
LDL				
T Cholesterol [a]	13.44±0.62	63.27±5.12*** (+4.70F)	48.77±2.62*** (-23)	43.72±4.00*** (-38)
Phospholipid [a]	12.64±1.00	44.12±2.87*** (+3.49F)	34.00±2.12** (-22)	29.14±2.17*** (-33)
Triglyceride [a]	15.28±1.00	35.17±2.61*** (+2.30F)	25.38±2.00*** (-27)	24.88±1.62*** (-29)
Apoprotein[b]	17.00±1.14	30.27±1.88*** (+1.78F)	23.00±1.00*** (-24)	21.00±1.60*** (-30)
HDL				
T Cholesterol [a]	46.38±4.00	36.17±2.40*** (- 22)	43.37±2.88* (+17)	44.00±3.16* (+18)
Phospholipid [a]	39.00±3.00	29.38±2.17*** (- 25)	33.80±2.14* (+13)	34.66±2.81* (+15)
Triglyceride [a]	16.14±1.00	12.00±0.78*** (- 26)	15.17±1.00** (+21)	16.00±0.79*** (+25)
Apoprotein[b]	170.33±13.62	122.62±10.14*** (- 28)	141.24±12.44* (+13)	150.39±14.00* (+18)
Plasma LCAT activity[c]	70.30±4.84	35.69±2.44*** (- 49)	50.31±3.82*** (+29)	52.77±5.00*** (+32)
PHLA[d]	18.00±1.17	11.00±0.62*** (-38)	14.00±0.79** (+21)	14.48±1.01*** (+24)

Units: (a.) mg/dl serum, (b) g/dL serum, (c). n mol cholesterol released /h/l plasma, (d.) n mol free fatty acid formed /h/ml plasma. Values are mean ± SD from six animals; ***P<0.001, **P<0.01, *P<0.05; Cholesterol treated compared with control, cholesterol and drug treated with triton only.

Table 3: Effect of andrographolide and Gemfibrozil on hepatic lipids and fecal bile acid excretion in hyperlipemic rats.

Parameters	Control	Cholesterol treated	Cholesterol and andrographolide treated	Cholesterol and Gemfibrozil treated
A Liver				
LPL activity[a]	132.22 ±10.60	73.30 ±5.69*** (-44)	83.66 ±8.00 (+12)	89.27 ±5.77* (+18)
Total cholesterol[b]	7.00 ±0.25	12.17 ±1.00*** (+1.73F)	9.11 ±0.37** (-25)	8.80 ±0.40*** (-28)
Phospholipid[b]	24.31 ±2.00	40.18 ±3.12*** (+1.65F)	28.66 ±1.60*** (-28)	26.92 ±2.00*** (-33)
Triglyceride[b]	11.23 ±0.77	16.68 ±1.10*** (+1.48F)	13.11 ±0.69** (-21)	12.00 ±1.00*** (-28)
Protein[b]	152.88 ±13.18	220.84 ±13.92*** (+1.44F)	178.80 ±14.42*** (-19)	165.50 ±14.00*** (-25)
B Faecal bile acids				
Cholic acid[c]	85.73 ±6.89	50.22 ±3.78*** (-41)	69.92 ±6.00*** (+28)	67.00 ±6.10*** (+25)
Deoxycholic acid[c]	55.77 ±5.00	25.10 ±2.00*** (-55)	38.80 ±3.00*** (+54)	44.89 ±3.12*** (+44)

Unit: a. n mole free fatty acid formed/h/mg protein, b. mg/g, c. µg/g
Values are mean ±SD of six animals; ***P<0.001, **P<0.01, *P<0.05. Cholesterol treated group compared with control.

Discussion

Andrographolides mixture from *A. paniculata* and Gemfibrozil both cause a significant decrease in the serum level of lipids in triton induced hyperlipidemic rats and this model has been successfully used for the evaluation of lipid lowering activity of andrographolides mixture in the rats.[19,20] The present investigation with HDF fed hyperlipidemic animals shows that andrographolides could increase the level of HDL by increasing the activity of LCAT, which play a key role in lipoprotein metabolism. The increase of the receptor mediated catabolism of LDL as well as the lipolytic activity in liver and the level of blood HDL-TC followed by the decrease of B-lipoprotein lipids and the suppression of hepatic lipids by these extract are of great utility for regressing atherosclerosis.[21] The stimulation of LDL catabolism by these extract in hyperlipidemic animals may be due to a significant decrease in the level of serum and tissue lipids. The compound may also enhance the synthesis of LDL apoprotein (Apo B) as well as receptor protein to accelerate the turnover of cholesterol. Increased synthesis of receptor protein decreased the rate of hepatic lipid synthesis and inhibition of oxidative modification in LDL may regulate the cholesterol level in the body.

Conclusion

Hyperlipidemia is one of the important risk factors involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart diseases are strongly associated with disorders of lipid metabolism and plasma lipoproteins. Triton WR-1339 treated rats are considered to be an useful acute hyperlipidemic model associated with inactive lipoprotein lipase.[22]. Triton WR-1339 acted as a surfactant to block the uptake of lipoprotein from the circulation by extra hepatic tissues resulting in an increase in the level of circulatory lipoproteins.[23] Andrographolides were found to inhibit HMG- Co A reductase activity.

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References

1. Chao WW, Kuo YH, Lin BF. Anti-inflammatory Activity of New Compounds from Andrographis paniculata by NF-κB

Trans-Activation inhibition, J Agric Food Chem, 2010; 58:2505-2512.

2. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from Andrographis paniculata, J Ethnopharmacol, 2004; 92: 291-295.

3. Geethangili M, Rao YK, Fang SH, Tzeng YM. Cytotoxic constituents from *Andrographis paniculata* induce cell cycle arrest in Jurkat cells.Phytother Res, 2008; 1336-1341.

4. Mosmann TR, Sad S. The expanding universe of T-cell subset: Th1, Th2 and More,Immunol Today, 1996; 17:138-146.

5. Reddy VL, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV, Rao TP, Ram TS, Gonnade RG, Bhadbhade M, Venkateswarlu Y. A new bis-andrographolide ether from Andrographis paniculata nees and evaluation of anti-HIV activity. Nat Prod Res, 2005; 19: 223-230.

6. Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, Well K, Wenner CA, Standish LJ. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother Res, 2000; 14:333-338.

7. Singha PK, Roy S, Dey. Protective activity of andrographolide and arbinogalactan proteins from *Andrographis paniculata* Nees. against ethanol induced toxicity in mice., J Ethnopharmacol, 2007; 111:13-21.

8. Zhang CY, Tan BK. Hypotensive activity of aqueous extract of *Andrographis paniculata* in rats. Clin Exp Pharmacol Physiol, 1996; 23:675-678.

9. Zhang CY, Tan BK. Mechanism of cardiovascular acrivity of *Andrographis paniculata* in the anaesthetized rat., J Ethnopharmacol, 1997; 56:97-101.

10. Hui H, Tang G, Go VLW. Hypoglycemic herbs and their action mechanisms. Chin Med, 2009; :11-14.

11. Chen JH, Hsiao G, Lee AR, Wu CC, Yen MH. Andrographolide suppresses endothelial cell apoptosis via activation of phosphatidyl inositol-3-kinase/Akt pathway.Biochem Pharmacol, 2004; 67:1337-1345.

12. Yu BC, Hung CR, Chen WC, Cheng JT. Antihyperglycemic effect of andrographolide in streptozotocin induced diabetic rats. Planta Med, 2003; 69:1075-1079.

13. Nagasaki, T and Akanuma, Y. A new colorimetric method for determination of plasma lecithin : Cholesterol acyl transferase activity. Clin. Chem. Acta 1997; 75:371- 375.

14. Wing, D.R. and Robinson, D.S. Clearing factor lipase in adipose tissue. Biochem. J., 1968; 109:841-849.

15. Burstein, M. and Legmann, P. Monographs on atherosclerosis in lipoprotein precipitation. Ed. by T. B. Clarkson, 1982; Vol 11: 76-83, S. Kagar, London.

16. Khanna, A.K., Chander, R., Singh, C., Srivastava, A.K and Kapoor, N. K. Hypolipidemic activity of *Achyranthes aspera* Lin. in normal and triton induced hyperlipidemic rats. Ind. J.Exp. Biol., 1992; 30:128-130.

17. Khanna, A.K., Chander, R., Singh, C., Srivastava, A.K and Kapoor, N.K. Hypolipidemic activity of *Terminalia chebula* in rats. Fitoterapia, 1993; 64:351-356.

18. Mosbach, E. H., Kienisky, H. J., Hal, P. and Kendall, F.E. Determination of deoxycholic acild and cholic acid in bile. Arch. Biochem. Biophys. 1954; 51:402-409.

19. Nityanand, S and Kapoor, N. K. Case history of guggulip. A hypolipidemic agent in proceeding of the fifth Asian Symposium on medicinal plants and species, Bangkok (eds, Han, B. H., Han, DS., Han,YN and Wox, W. S., 1984; 171-182.

20. Khanna, A.K., Chander, R.,Kapoor, N.K. and Dhawan, B.N. Hypolipidemic activity of Picroliv in albino rats. Phytother. Res., 1994; 8:403-407.

21. Chander, R., Khanna, A.K. and Kapoor, N. K. Lipid lowering activity of Guggulsterone from *Commiphora mukul* in hyperlipidemic rats. Phytother. Res., 1996; 10:508-511.

22 Xie, W., wang, W., Su, H., Xing, D., Cai, G. and Du,L. Hypolipidemic mechanisms of *Ananas comosus* L. leaves in mice: different from fibrates but similar to statins. J. Pharmacol. Sci. , 2007; 103:267-74.

23. Umesh, K. P., Saraf, S. and Dixit, V. K. Hypolipidemic activity of seeds of *Cassia tora* Linn . J. Ethnopharmacol, 2004; 90: 249-52.