



Lipid lowering activity of *Abutilon indicum* (L.) leaf extracts in rats

Ranjan Kumar Giri^{*1}, Sunil Kumar Kanungo¹, V.Jagannath Patro², Sujit Dash¹, Durga Charan Sahoo³

^{*1}Department of Pharmacology, Institute of Pharmacy and Technology, Salipur, Cuttack – 754202, India

²Department of Pharmachemistry, College of Pharmaceutical Sciences, Mohuda, Berhampur, Orissa, India

³Department of Pharmacognosy, Dadichi College of Pharmacy, Vidya Vihar, Cuttack, Orissa, India

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ABSTRACT

Lipid lowering effect of the successive extracts of the leaf of *Abutilon indicum* (L.) was evaluated in triton and diet induced hyperlipidemic models of wistar albino rats. The ethanolic and water extract at 400mg/kg dose levels inhibited the elevation in serum cholesterol and triglyceride levels on Triton WR 1339 administration rats. The extracts at the same dose level significantly attenuated the elevated serum total cholesterol and triglycerides with an increase in high-density lipoprotein cholesterol in high-fat diet-induced hyperlipidemic rats. The standard dose Atrovastatin in the former and Gemfibrozil in the later studies showed slightly better effects.

Keywords: *Abutilon indicum*, Antihyperlipidemic activity, High-fat diet, Triton WR 1339, HDL-C, Triglycerides

INTRODUCTION

Hyperlipidemia (elevated levels of triglycerides or cholesterol) and reduced high- density lipoproteins (HDL-C) occur as a consequence of several interrelated factors that may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism¹. Elevated serum concentrations of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) appear to increase the risk of individual in developing coronary heart disease (CHD)². Lipid lowering therapy is indicated in primary and secondary prevention of cardiovascular diseases in addition to the management of all other risk factors including smoking, diabetes and obesity³. The current antihyperlipidemic therapy includes principally statins and fibrates. The former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and later acts by enhancing the clearance of triglyceride rich lipoproteins¹. The investigation of lipid lowering activity on herbs will be useful strategy in the discovery of new lead molecules eliciting improved activity by regulating through different mechanism of action. The plant extracts maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology.

Abutilon indicum L. of family Malvaceae is found throughout tropical and sub tropical region in India, is known as, Atibal in Sanskrit. The various parts of plant have claimed to have several

traditional medicinal properties. Traditionally the plant is used in inflammation, piles, gonorrhoea treatment and as an immune stimulant. Root and bark are used as aphrodisiac, anti diabetic, nervine tonic, and diuretic. Seeds are used as aphrodisiac and in urinary disorders⁴. The plant is reported to have analgesic⁵, hypoglycemic^{6,7}, and hepato protective activity⁸. Isolation of sesquiterpine lactone, Gallic acid and eugenol had been reported in the literature⁹. The present study is an attempt to validate lipid lowering activity of *Abutilon indicum*.

MATERIALS AND METHODS

Plant material: *A. indicum* leaves were collected from the local area of Salipur, Cuttack, Orissa, during January-February and was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Tambaram, Chennai, India, and a voucher specimen holding No.PARC/2007/25, was deposited in the same center. The air dried leaves were extracted with Petroleum ether (60-80°C), CHCl₃, EtOH, and water successively by using Soxhlet apparatus and the yields were found to be 2.25, 4.38, 14.2 and 9.92%, respectively, on dried weight. Phytochemical screening¹⁰⁻¹² gave positive test for steroids and triterpenoids in petroleum ether extract; steroids, flavonoids, and tannins in CHCl₃ extract; triterpenoids, flavonoids, tannins, and glycosides in EtOH extract; saponins, flavonoids, and glycosides in water extract.

Chemicals and reagent: Triton WR 1339 (Sigma USA) were from commercial sources. Serum Cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were determined by using the kits of Qualigens fine chemicals. Atrovastatin and Gemfibrozil were obtained from DR. Reddy's Lab, Hyderabad and Sun pharmaceuticals, India respectively.

Animals: Male wistar strain albino rats (150-250g) were obtained from Central Animal House of Institute of Pharmacy and Technology

***Corresponding author.**

Ranjan Kumar Giri

Department of Pharmacology

Institute of Pharmacy and Technology,

Salipur, Cuttack – 754202, India

Tel.: + 91-9861512071

Telefax: +91-671-2351918

E-mail: ranjanrifampin@gmail.com

Table 1- Constituents of high fat diet

Ingredients	Quantity(g/100g)
Cornflour	25
Milk powder	15
Sucrose	15
Casein	5
Egg yolk	3
Lard	35
Salt mixture	1
Cholesterol	1

Table 2: Effects of the various extracts of *A. indicum* leaves on lipid profile in triton induced study

Group	Total Cholesterol(mg/dl)		Triglycerides(mg/dl)	
	0 hr	24 hr	0hr	24 hr
Control	47.0±3.2	182.3±4.2	73.3 ±5.5	356.2 ± 24.75
Pet-ether	52.5±2.7	176.4±3.2	68.5±2.4	312.4 ± 28.66
CHCl ₃	45.3±2.9	174.0±2.6	72.2±3.1	305.5 ± 26.34
EtOH	48.7±1.3	146.4±3.2*	66.5±7.9	235.4 ± 21.66*
Aqueous	45.6±2.3	135.5±5.2*	65.3±5.9	227.3± 24.67*
Atrovastatin	49.5±2.7	118.6±4.3*	68.3±2.4	214.3±21.42*

Values are mean ± SE of 6 rats in each group* - P<0.001 compared with vehicle (untreated) control

Table 3: Effects of the various extracts of *A. indicum* leaves on lipid profile of hyperlipidemic wistar rats in diet-induced hyperlipidemia.

Group	Total cholesterol(mg/dl)			Triglycerides(mg/dl)			Atherogenic index (total cholesterol/HDL-C)		
	Normal value	On induction of hyperlipidemia		Normal value	On induction of hyperlipidemia		Normal value	On induction of hyperlipidemia	
		0 th day	7 th day		0 th day	7 th day		0 th day	7 th day
Control	51.6±2.6	72.1±3.1	78.6±2.9	69.8±2.9	132.6±2.1	173.6±4.3	3.65±0.23	3.87±0.12	5.28±0.27
Pet-ether	49.8±2.3	75.6±1.8	72.8±4.3	71.7±2.3	134.8±2.6	165.3±3.1	3.37±0.20	4.13±0.08	3.02±0.07
CHCl ₃	49.3±2.1	76.3±1.5	70.2±1.2	68.8±3.7	136.2±2.3	161.2±2.1	3.17±0.32	4.12±0.17	2.46±0.08
EtOH	48.3±2.4	75.2±2.7	52.6±1.6*	66.3±2.8	135.4±2.9	96.8±2.7*	3.23±0.26	4.36±0.17	2.75±0.28*
Aqueous	48.3±2.2	73.2±2.6	51.6±1.4*	63.3±3.2	130.4±2.7	90.8±2.7*	3.21±0.26	4.16±0.17	2.25±0.28*
Atrovastatin	49.3±3.1	75.6±2.5	49.6±1.8*	65.3±3.6	133.84±2.6	88.7±2.7*	3.35±0.29	4.06±0.19	2.08±0.26*

Values are mean ±SE of 6 rats in each group* - Represent values significantly different in paired t-test as compared to 0th day values (P<0.01)

Salipur, Cuttack, Orissa India. The animals were housed under standardized environmental conditions (at normal room temp, with a 12 hour light and dark cycle) and fed with standard pellet chow feed and water *ad libitum*. The animal protocol was approved by Institutional Animal Ethical Committee of I. P. T., Salipur, Cuttack, Orissa, India with registration number 1053/ac/07/CPCSEA. All the experiments were performed as per the CPCSEA guidelines.

Triton induced hyperlipidemic study¹³

The use of Triton WR 1339 induced hyperlipidemia through accelerated hepatic cholesterol synthesis was suggested as an important approach to screen the action of hypolipidemic drugs (Paoletti, 1962)¹⁴. Male wistar rats weighing 200-250g were divided into 4 groups of 6 animals each. Group-1(Vehicle control) received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week. Group 2-5 received pet. ether, CHCl₃, EtOH and aqueous extracts respectively at the dose of

400mg/kg body weight and Group-6 received Atrovastatin 1mg/kg body weight once daily for one week. On seventh day, 200mg/kg Triton WR 1339 (isooctyl polyoxyethylene phenol) was injected (ip), to all the six groups of rats immediately after drug administration. Serum total cholesterol and triglycerides were estimated for individual animals in autoanalyser (Microlab 100) on seventh day previous to drug treatment and after 24 hr of Triton administration. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes and serum was separated in cooling centrifuge (Remi, C24) by centrifuging at 2500 rpm for 10 min. The observations made were recorded in Table-2.

High-fat diet-induced hyperlipidemic study¹⁵

Hyperlipidemia was induced in male wistar rats weighing 150-180g by feeding them with a high fat diet, (Table 1) for 4 weeks. High-fat diet increased the serum cholesterol and triglycerides to about 75-80% of the normal levels and reduced the HDL-C levels significantly (Table-3). The rats with significantly higher values of serum cholesterol and triglyceride values compared to that of normal animals were considered to be hyperlipidaemic and six hyperlipidaemic animals were grouped for one treatment. Group 1-received 0.3% w/v CMC and served as vehicle control, Group 2-5 received pet.ether, CHCl₃, EtOH and aqueous extracts respectively at the dose of 400mg/kg body weight. Hyperlipidaemic animals of sixth group were administered with the standard drug Gemfibrozil 50mg/kg body weight for one week.

All the six groups were kept on the same high fat diet throughout drug treatment. Serum total cholesterol, triglyceride and HDL-C of the non-fasted animals were estimated on seventh day after 1hr of dosing. Atherogenic index was calculated using the formula:

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol}}{\text{HDL-C}}$$

Statistical analysis

Data are represented as mean ±SEM (Standard error of mean). The group means were compared for significant difference (p<0.01) by Student's t test in triton model and paired t-test in diet model.

RESULTS AND DISCUSSION

The systemic administration of the surfactant Triton to rats resulted

in an enormous elevation of serum cholesterol and triglycerides at 24 hr (Table 2). The pet-ether, CHCl₃, EtOH and aqueous extracts of *A. indicum* inhibited the highly significant elevation in cholesterol by 3.34, 4.77, 24.52, 34.53 and 53.70% at 400 mg/kg dose levels, respectively as compared to that of untreated vehicle control group. Similarly the above successive extracts of *A. indicum* lowers the Triglycerides levels 14.02, 16.59, 51.40, 56.70, 66.21% respectively in comparison to that vehicle control rats (Table 2). Atrovastatin, the lipid controlling mechanism of which is inhibition of synthesis of cholesterol in the liver, was employed as the standard drug in Triton induced model. The treatment with Atrovastatin resulted in a slightly better effect than *A. indicum*. These results indicate that the extracts of *A. indicum* may interfere with cholesterol biosynthesis as Triton accelerates the hepatic synthesis of cholesterol¹⁶.

Triton induced hypercholesterolaemia, though simple and rapid for evaluating hyperlipidemic compounds, is rather artificial. Hence the lipid controlling potential of *A. indicum* leaf was further validated in diet-induced hyperlipidemic rat model. When male wistar albino rats were kept on high-fat diet supplemented with 1% cholesterol for 4 weeks, there was elevated serum cholesterol levels and triglyceride levels were almost doubled whereas, HDL-C levels were reduced significantly as indicated by low value of atherogenic index (Table 3). Elevated circulating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis¹⁷. The treatment of hyperlipidemic rats with the ethanolic and aqueous extracts of *A. indicum* for one week, significantly brought down the elevated serum total cholesterol and triglycerides improving the HDL-C levels as shown by reduced atherogenic index (Table 3). Similar to Gemfibrozil (50mg/kg) the standard fibrate drug used, the extract may have enhanced the breakdown of lipids, thus modifying the altered lipid metabolism induced by high fat-diet. Increase in HDL levels and reduction in LDL shows the intensive conversion of LDL to HDL and clearance of circulating lipids. Total cholesterol/HDL-C ratio of > 4.5 is associated with increased coronary heart disease (CHD) risk and the ideal ratio is =3.5¹⁸. A significant reduction in the atherogenic index by ethanolic and aqueous leaf extracts of *A. indicum* treatment demonstrates the protective efficacy of these extracts against atherogenesis. Consequently the lipid regulating efficacy of the EtOH and aqueous leaf extracts of *A. indicum* leaf would be beneficial in the prevention of plaque formation leading to atherosclerosis and CHD accelerated by high fat diets.

The lipid lowering activity of the EtOH and aqueous leaf extracts of *A. indicum* may be attributed to the phytoconstituents present, such as triterpenoids, flavonoids, tannins, glycosides, and saponins in it, as reported for other plant extracts¹⁹⁻²¹. Saponin derived from *Medicago sativa* were reported to reduce blood cholesterol by competing with cholesterol at binding sites or interfering with cholesterol biosynthesis in the liver²². Phenolic active principle present in *Anethum graveolens* were observed to be responsible for lowering TC and LDL-C and elevating HDL-C in hypercholesterolaemic rats²³.

The findings of the study reveals that ethanolic and aqueous extracts of *Abutilon indicum* L. leaf can effectively control the blood lipid levels in dyslipidaemic conditions by interfering with the biosynthesis of cholesterol and utilization of lipids.

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