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ARTICLE

Hypolipidemic effect of *Terminalia arjuna* (L.) in experimentally induced hypercholesteremic rats

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ABSTRACT The hypolipidemic activity of the 50% ethanol extract of bark of *T. arjuna* were evaluated in rats. The 50% v/v ethanol bark extract at the dose of 40mg/kg body weight, substantially reduced the plasma total cholesterol, triglycerides and LDL cholesterol while HDL cholesterol increased in experimental group in comparison with hypercholesterolemic animal group. Atherogenic index and liver weight of treated animals also showed significant decrease. A significant increase in the activities of lipoprotein lipase and plasma LCAT enhanced hepatic bile acid synthesis and thereby increased degradation of cholesterol to neutral sterols. Furthermore, the activities of lipogenic enzymes like HMG-CoA reductase, glucose-6-phosphate dehydrogenase and malate dehydrogenase were significantly reduced. The bark extract of *Terminalia arjuna* has excellent hypolipidemic activity. The effect of the extract seems to be mediated through increased hepatic clearance of cholesterol, down regulation of lipogenic enzymes and inhibition of HMG- CoA reductase.

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KEY WORDS

atherogenic index
lipid lowering
Terminalia arjuna
neutral sterols
plasma LCAT

Coronary heart disease (CHD) is the main cause of death in western countries and Asia. Among CHDs, ischemic heart disease (IHD) leads to the highest mortality rate. The number of heart patients suffering from IHD worldwide is gradually increasing. About 41% deaths in United States are due to the heart diseases (Dallas 2001). It is well known that the three major risk factors for IHD are hypercholesterolemia; hypertension and smoking. Extensive epidemiological studies have shown that increased blood cholesterol level is a major cause of coronary heart diseases (Gambhir et al. 2001). Studies have also demonstrated the relationship between plasma cholesterol levels and the development of IHD. Hypercholesterolemia is generally, associated with an increase in plasma concentration of LDL and VLDL. Lowering of elevated levels of LDL cholesterol can slow the progression of atherosclerotic lesions (Altschul, 1964). To reduce the risk associated with high serum cholesterol levels, the development of several hypolipidemic drugs and therapies have been adopted intensively in India and other countries. About 70% of total cholesterol in human is synthesized *de novo* and the remaining is supplied by absorption from diet (0.3- 0.5 g/day in human). Several methods are presently practised to control blood cholesterol levels. These include balance of dietary fats; bile acids sequester and use of HMG-CoA reductase inhibitors (statins). HMG-CoA reductase is the key enzyme in the cholesterol biosynthesis pathway and its inhibition has proven to be the most efficient therapy for managing hypercholesterolemia (Alberts et al. 1999).

Many plants in the Indian system of medicine have been reported to be beneficial in hypercholesterolemia. Extracts of various parts of plants like *Allium sativum*, *Allium cepa, Cucurma longa, Emblica officinalis, Picrorrhiza kurroa* etc. are known to have antihyperlipidemic activities (Anonymus, 1999). *Terminalia arjuna* commonly known as *Arjuna* is a large deciduous tree found all over in India and Himalaya (Dwivedi and Udapa 1989). The bark of *T. arjuna* has been recommended and used as cardiac tonic and bark powder/ decoction is used to treat heart diseases, bone fractures, skin diseases, polyuria, white discharge, giddiness, fever and worms (Anonymus, 1999). In the present study, the ethanol extract of *T. arjuna* bark was investigated for hypolipidemic activity in experimental animals and some possible mechanisms of its lipid lowering properties are discussed.

Materials and Methods

Plant material

Fresh *Terminalia arjuna* bark was obtained from the Toranmal forest of Nandurbar district, Maharashtra, India, in the month of January 2007. The plant was authenticated by Dr. D. A. Patil, Botanist, S.S.V.P.S. College, Dhule, India and a voucher specimen (No. 094) has been deposited in the herbarium of the Department of Pharmacognosy, R.C. Patel College of Pharmacy, Shirpur

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Table1. Effect of 50% ethanol extract of *T. arjuna* on lipid profile of hypercholesterolemic rats.

Animal Groups	Parameters				
	TC (mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
Group A (Control)	68.20±6.22	89.32±3.14	34.82±5.47	16.34±2.56	15.40±1.23
Group B (Hypercholesterolemic)	149.20±9.36*	209.10±16.23*	37.12±7.58*	70.26±8.16	40.00±7.63*
Group C (Hypercholesterolemic plus 50% extract)	70.12±5.95#	90.02±2.56#	35.40±4.25#	16.68±2.56	15.22±3.10#
Group D standard (Atrovastatin 5mg/kg BW)	68.90±5.21	88.32±3.14	34.00±3.89	17.24±3.26	13.19±3.44

Values are expressed as Mean ± SD of each group (*n=6*). The symbols * and # represents statistical significance vs. hypercholesterolemia (Group B) and standard group (Group D) respectively $p< 0.05$.

Preparation of plant material

The shade dried *T. arjuna* bark was coarsely powdered and 200g of coarse powder of 20-40 mesh size was refluxed with 50% v/v ethanol for three hours using Soxhlett's apparatus. The extract was filtered and evaporated in a vaccum evaporator (Buchi, Switzerland) until a gel like residue remained. The amount of residue remained was measured and stored in glass bottle at 4°C and was redissolved in physiological saline at the time of use.

Animal studies

Twelve week old 24 healthy male albino Wistar rats weighing 150 – 160 g each were used. The animal experiments were conducted at the animal house of R. C. Patel College of Pharmacy, Shirpur, India as per internationally accepted principles for laboratory animal use and approved by the Institutional Animal Ethics Committee. All animals were maintained at 27±1°C with 12 h light and dark cycle. The animals were fed with standard diet and water *ad libitum*. Rats were divided in to four groups (*n=6*). Their body weight was monitored on 3 day interval. Group A rats were served as non- treated control and received standard diet plus 1ml saline for six weeks, group B received high fat diet (athero diet) for six weeks whereas group C rats received high fat diet plus 10, 20, 30, 40 and 50mg/kg body weight extract of *Terminalia arjuna* for six weeks. Group D rats received athero diet plus standard atrovastatin tablet at 5 mg each (Alberts et al. 1990). The atrovastatin was used as a reference compound to compare the hypolipidemic effect of the extract. The extract was administered orally through gastric intubation. High fat diet composed of (g/100 g of normal diet) hydrogenated sunflower oil: 20, egg yolk: 35 and cholesterol: 0.5 (Gandhi et al. 1992). After 6 weeks treatment, blood samples were collected by retro orbital sinus and serum was separated.

The acute oral toxicity study

The assay was performed as per the OECD guidelines (OECD-TG, 425, 2002) with albino rats. The age, body weight, acclimatization, randomization, accommodation, environmental condition for the animal during the period of study was as per the standard OECD guidelines. The limit dose of 2000mg/kg body weight of the animal was administered to the test animals (*n=6*) orally through gastric intubation.

Biochemical analysis

The serum and liver tissues were extracted as per the procedure described by Menon et al. (1976). Analysis of serum for total cholesterol (Zlatkis et al. 1953), triglycerides (Gottfried and Rosenberg 1973), and HDL cholesterol (Burnstein et al. 1970) was performed by micro titer plate reader using standard kits (Span Diagnostics, Surat, India). Serum LDL cholesterol concentration was determined using Friedwald formula (Friedwald et al. 1970).

Bile acids and fecal sterols were extracted as per the previously known procedures (Grundy et al. 1965). The bile acids were extracted from liver according to the procedure of Okishio et al. (Okishio et al. 1967). Total bile acids (Palmer 1969), neutral sterols (Ghanbari and Leelamma 1996) and serum lipoproteins (Warnick and Albers 1978) were also estimated. The activity of plasma lecithin cholesterol acyl transferase (LCAT) (EC 2.3.1.43) and lipoprotein lipase was estimated as described earlier (Annie and Kurup 1986). The extent of increase in the cholesterol ester/unesterified cholesterol ratio was taken as a measure of LCAT activity. Activity of glucose -6-phosphate dehydrogenase and malic enzyme was determined as per previously used methods (Annie and Kurup 1986). β hydroxy β methyl glutaryl CoA reductase (HMG CoA reductase EC 1.1.1.34) activity was assayed using the method described earlier (Rao and Ramkrishnan, 1975). The ratio of HMG CoA to mevalonate was taken as an index of enzyme activity which catalyzes the conversion of HMG to mevalonate. The lower the ratio, the higher the enzyme activity (Chitraand Leelamma 1997).

Atrovastatin

The standard atrovastatin tablets were procured from Lupin Pharmaceuticals Limited, Tarapur, India.

Table 2. Effect of 50% ethanol extract of *T. arjuna* on cholesterol deposition, body weight and atherogenic index of hypercholesterolemic rats.

Animal Groups	Parameters		
	Relative liver weight (gm)	Atherogenic index	Body weight (gm)
Group A Control	5.12±1.85	0.91±0.09	287
Group B Hypercholesterolemic	6.90±1.10*	2.97±0.12*	365
Group C Hypercholesterolemic plus 50% extract	5.07±1..93#	0.90±0.08#	290
Group D standard Atrovastatin (5mg/kg BW)	4.90±1.23	0.89±0.02	304

Values are expressed as Mean ± SD of each group (*n=6*). The symbols * and # represents statistical significance vs. hypercholesterolemia (Group B) and standard group (Group D) respectively $p< 0.05$

Statistical analyses

Statistical significance of data was analyzed using one-way analysis of variance (ANOVA) in Microsoft Excel. Each data value is expressed as the mean ±S.D.of six animals. Statistical differences were considered significant at $p< 0.05$.

Results

The choice of the solvent (50% ethanol) for extraction was based on literature reports on immunomodulatory and antioxidative ((Dwivedi 2007) properties of ethanol extracts of *T. arjuna* bark. The yield of the ethanol extract was 8.5%. Primary phytochemical screening of the ethanol extract of *T. arjuna* bark revealed the presence of triterpenoids, phenols, flavonoids, tannins and saponins in agreement with a previous report (Dwivedi 2007).

Preliminary studies with different doses of the ethanolic extract (10, 20, 30, 40 and 50 mg/kg body weight of rats) indicated a dose dependent decrease in total cholesterol in group C (induced hypercholesterolemic) rats till 40mg/kg body weight dose. The decline in the total cholesterol beyond this does became independent of the dose and was not significant and hence all other parameters were evaluated for animals treated with this dose.

It was observed that keeping the animals on high fat diet significantly increased the total cholesterol (TC), triglycerides (TG), LDL and VLDL cholesterol as compared to the rats on normal diet. Co-administration of bark extract of *T. arjuna* at 40mg/kg body weight resulted in considerable decline in the levels of these parameters. The lipid profile of different groups of animal after the experiment period of 6 weeks is presented in Table 1. The levels of serum TC, TG, VLDL and LDL- cholesterol in the group C animals were significantly lower than group B (animals receiving high fat diet only) and comparable to group D (animals receiving high fat diet plus Atrovastatin at 5mg/kg body weight). On the other hand, serum HDL-cholesterol concentration in group C animals was marginally higher than that of other groups (Table1). The extract of *T. arjuna* at the dose of 40mg/kg body weight lowered the plasma total cholesterol, LDL and TG by 53%, 76.25% and 57% ($p< 0.05$), respectively in group C *vis- a- vis* group B animals and the values were comparable with that of group A and group D.

The liver weight, atherogenic index and body weight in different groups is presented in Table 2. The liver weight in treated group (group C) was significantly lower than that in hypercholesterolemic group (group B) indicating decrease in cholesterol and fat deposition in liver (Table 2). Atherogenic index in group C was three times less than group B and was comparable to group A and D. The body weight of the group B animals showed significant weight gain when fed with high fat diet over the group A animals (normal controls). Treatment with bark extract and standard drug (Atrovastatin) (Alberts et al. 1989) in group C and group D, respectively reduced body weight by approximately 20% compared to group B animals.

The activity of glucose -6-phosphate dehydrogenase, malate dehydrogenase and, HMG-CoA reductase was significantly reduced while activities of plasma LCAT and lipoprotein lipase were enhanced in the animal group fed with the extract (Table 3). The high concentrations of fecal neutral sterols and bile acids in the liver in the treatment group shows the enhanced rate of degradative processes and reduction in intestinal absorption of free cholesterol and other lipids. The significantly higher levels of bile acids in the liver and feces and increased concentrations of fecal neutral sterols provide the evidence for higher rate of degradation.

The acute and oral toxicity studies provide information on health hazards likely to arise from the short term exposure to the test substance by oral route. The results of the toxicological studies showed that the administration of ethanolic extract of *T arjuna* bark by oral route at the limit dose (2000mg/kg

Table 3. Concentration of hepatic and fecal bile acids and sterols, activities of lipogenic and lipolytic enzymes in rats fed with 50% ethanol extract of *T. arjuna*.

Parameters	Animal groups			
	Group A (Control)	Group B (Hypercholesterolemic)	Group C (Hypercholesterolemic plus 50% extract)	Group D (Standard Atrovastatin)
Hepatic bile acids (mg/100 g)	36±2.3	40.6±3.3	70.6±1.5#	39.2±2.2
Hepatic neutral sterols (mg/100 g)	100.3±3.5	105.6±4.5	135.2±3.2#	105.5±5.2
Fecal bile acids (mg/rat/day)	35.9±1.3	38.3±2.5	51.3±2.1#	40.3±3.2
Fecal neutral sterols (mg/rat/day)	90.5±2.6	98.8±3.1	123.5±3.2#	100.2±3.1
Plasma LCAT[A]	28.5±0.6	27.9±0.3	39.4±0.8#	30.2±0.5
Lipoprotein lipase[B]	40.6±1.2	35.3±1.2	56.6±1.6#	45.3±1.2
Glucose-6-phosphate dehydrogenase[C]	64.2±1.3	100.3±3.2	62.2±1.2#	60.1±2.0
Malate dehydrogenase[D]	501.3±8.6	990.3±9.6	453.2±9.6#	503.2±8.3
HMG CoA reductase (ratio of HMG CoA to mevalonate)[E]	4.1±0.2	6.8±03	3.9±0.3#	2.1±0.2

Control group (A) and group B compared with 50% ethanol extract fed group. # $p < 0.05$ [A] % increase in the ratio of ester cholesterol to free cholesterol during incubation [B] µ moles of glycerol liberated /h/g protein [C] One unit is defined as that amount of the enzyme that causes an increase of 0.01in optical density/mg protein. [D] One unit is defined as that amount of the enzyme that causes an increase of 1.0 in optical density/mg protein (Sudheesh et al.1997). [E] Decreased ratio indicates increased activity (Chitra and Leelamma 1997).

body weight) did not produce any signs of toxicity or deaths in experimental animals indicating minimal or no chance of toxicity of the extract at the likely therapeutic dose in human which are lower by several orders of magnitude then no observed adverse effects level (NOAEL).

Discussion

The male albino wistar rats used in the present study were reported as ideal hypercholesterolemic models in previous studies (Mary et al. 2003). The decrease in the cholesterol levels of animals fed with extract may be attributed to increase in the level of serum HDL, increase in the activity of lipoprotein lipase and plasma LCAT, which are known to involve in transport of tissue cholesterol to liver for its excretion. Hence the hypocholesterolemic effect of the extract seems to be mediated through increased hepatic clearance of cholesterol, down regulation of lipogenic enzymes like glucose-6-phosphate dehydrogenase and malate dehydrogenase and cholesterol biosynthetic enzyme HMG-CoA reductase.

It is known that for being effective antihyperlipidemic agent the compound should reduce the plasma levels of LDL cholesterol as it transports 70% of plasma cholesterol in humans. Epidemiological and clinical studies have demonstrated positive correlation in LDL cholesterol level in serum and risk of coronary heart diseases (Kannel et al. 1971). The present report demonstrates significant decrease in plasma LDL-cholesterol level (quantitatively the most important lipoprotein class in control of serum cholesterol level) as a function of treatment by 50% alcoholic bark extract of *T. arjuna* in experimental animals. The reduced triglyceride level in treated animals could be co-related to elevated lipoprotein lipase activity in agreement with the previous report on a mushroom extracted exo-biopolymer (Sugiyama et al. 1995). The treatment may have an inhibitory effect on cholesterol deposition in liver tissues apparently by inhibition or down regulation of HMG-CoA reductase activity resulting in observed lesser relative liver weight in group C animals. The mechanism of hypolipidemic effect of *T. arjuna* is yet to be resolved. However, the previous report of Sinha et al. (2008) suggests that, the hypolipidemic activity may be attributed to inhibition of oxidative stress. Recent finding on Arjunic acid revealed its free radical scavenging potential (Sun et al. 2008). It appears that combination of more than one factor i.e. inhibition of HMG- CoA reductase activity, accelerated fractional turnover of LDL etc. may be responsible for the observed effect (Gandhi et al. 1992). Further the bark of *T. arjuna* is rich in glycosides, alkaloids and saponins and the glycosides are known for their cardioprotective activity (Dwivedi and Udupa 1989). The decrease in the serum total cholesterol concentration may be due to increase in HDL cholesterol which normally facilitates catabolism of excess cholesterol. Comprehensive chemical and pharmacological studies would help discover the exact mechanism of hypolipidemic effect of this indigenous tree and pave the way for its large scale commercial use as a cardio protective/curative drug.

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