Research Article

Evaluation of Hypolipidemic Activity of Various Phytoconstituents from *Terminalia arjuna* (Roxb. *ex* DC.) in Rat Fed with High Fat Diet

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ABSTRACT

The present study was designed to investigate the hypolipidemic effect of various phytoconstituents of T. Arjuna in rats fed with high fat diet. Based on the docking analysis revealed that the four phytoconstituents from T. Arjuna (quercetin, gallocatechin, luteolin, terminoside A) has been selected for the hypolipidemic activities. All these four phytoconstituents from T. arjuna (quercetin, gallocatechin, luteolin, terminoside A) were administered in doses of 15mg/kg/day to rats fed with high fat diet to assess its possible lipid-lowering potential. There was a noticeable increase in the body weight in HFD fed group ($p<0.001$), which was reduced by the administration of phytoconstituents from T. arjuna (15mg/kg). The elevated levels of total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C were observed in rats fed with high fat diet (group II). After the treatment of phytoconstituents from T. arjuna (quercetin, gallocatechin, luteolin, terminoside A) (15mg/kg/day) showed a significant ($p<0.001$) decrease in body weight, plasma total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C along with an increase in HDL-C when compared to HFD rats (group II). Hypolipidemic activity of the phytoconstituents from T. arjuna (quercetin, gallocatechin, luteolin, terminoside A) in chronic hyperlipidemic rats validates it is used traditionally as a part of folklore medicine in India, though there is no scientific evaluation to date.

Keywords: T. Arjuna, high fat diet, rats, quercetin, hypolipidemia

INTRODUCTION

Cardiovascular disease is leading cause of death in India as well as in western countries. Hyperlipidemia is one of the major causes of the development of cardiovascular disorders[1].In developing countries, the incidence of cardiovascular disease is increasing alarmingly. India is on the verge of a cardiovascular epidemics[2,3] The circulatory system disorders are going to be the greatest killer in India[4] .Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases[5]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death[6]. Hyperlipidemia characterized by elevated serum total cholesterol, low density, very low density lipoprotein cholesterol and decrease high density lipoprotein are the risk factor for coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease[7]. Among these hypercholesterolemia and hyper triglyceridemia are closely related to ischemic heart disease[8]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease[9]. Currently available hypolipidemic drugs have been associated with number of side effects[10]. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function[11]. Medicinal plants are used for various research purposes. It has been reported that traditional systems have immune potential against various diseases. More than thirteen thousand plants have been studied for various pharmacological properties.

The quest for finding the new safe and effective drug for dyslipidaemia in order to protect against cardiovascular disease (CVD) is going to be a continuous process amongst the scientific fraternity. Herbs have been used as food and for medicinal purposes for centuries. Research interest has been focused on various herbs possessing hypolipidemic property to reduce atherosclerosis that may be useful adjuncts in helping reduce the risk of CVD. Apart from the synthetic modern drugs like clofibrates, statins, there are efforts to find out herbal drugs possessing lipid lowering activities. Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects[12, 13].

The stem bark of *Terminalia Arjuna* (Roxb. *ex* DC.) Wight and Arn, of family Combretaceae is usually utilized by the specialists of Ayurveda for different cardiovascular sicknesses[14]. A few mixes of organic criticalness have been confined from the stem bark of *T. arjuna*, which incorporate (i) triterpenoids, for example, arjunolic corrosive, arjunic corrosive, arjunetin, arjunolitin (ii) tannins such as pentagalloyl glucose, hexadrox diphenyl galloyl glucose, tetragalloyl glucose, and ellagic acid (iii) flavonoids, for example, leucocyanidin and luteolin[15].

Numerous reports have detailed its antihypertensive, cancer prevention agent and hypocholesterolaemic[16, 17] impacts. Every one of these impacts has remedial vastness in cardiovascular illnesses of people. Hence, the objective of the present study was to investigate the hypolipidemic activities of various phytoconstituents from *Terminalia arjuna* (Roxb. *ex* DC.) in rat fed with high fat diet.

MATERIAL AND METHODS

Protein Preparation

The X-ray crystal structure of human HMGCR was downloaded from Protein Data Bank (http://www.rcsb.org) (PDB ID: 1HW8). Both chains A and B comprising the binding site of HMGCR inhibitor were considered. The protein is prepared using the protein preparation wizard in Maestro v9.2 by adding H-atoms, correcting bond orders, removing water molecule, and then adding missing residues/side chains to the target protein using Prime module (Schrödinger LLC 2011). Finally, protein was minimized at a pH of 7.4±2.0 using OPLS 2005 force field.

Ligand selection and preparation

In the current study, 23 chemical constituents reported from stem bark of Terminalia arjuna[18] (Table 1) were retrieved from pubchem database, and drawn using chemdraw tool, and then processed using LigPrep wizard in Maestro v9.2 (Schrödinger, Inc) The ligands were prepared using the Optimized Potentials for Liquid Simulations (OPLS) 2005 force field at pH 7.0± 0.4 and resulted in 72 pose generation.

Docking-based virtual screening (DBVS)

For carrying out the docking-based virtual screening (DBVS), Glide extra precision (XP) protocol (Schrödinger LLC 2011). Implemented in Maestro v9.2 was used. For this purpose, the active site residues GLU 559A, SER565A, LYS735A, HIS752A, ASN755A, LEU853A, ALA856A, LEU859A, LEU862A, ARG590B,SER684B, ASP690B, LYS691B, LYS692B present in the target protein (1HW8)[19] was used to generate the grid box around the active site, with default parameters. The prepared ligands using Ligprep were docked to the active site using the Glide XP default parameters. Glide XP ranked the docked conformation of the ligands according to their Glide G-score. The top nine Glide G-scoring compounds were selected as hits for the target protein. ΔG binding free energy was calculated for these hits using Prime MM/GBSA method.

Phytoconstituents from *Terminalia arjuna* (Roxb. *ex* DC.)

In order to investigate the binding capacity of a few important bioactive compounds of *Terminalia arjuna* stem bark with on HMG-CoA reductase enzyme protein in humans, we docked each compound to the target proteins. Computational docking studies of *T. arjuna* stem bark compounds with HMG-CoA reductase showed binding interaction with key residues.

Based on the docking analysis revealed that the four phytoconstituents from *T. arjuna* (quercetin, gallocatechin, luteolin, terminoside A) has been selected for the hypolipidemic activities.

Evaluation of Hypolipidemic activity

Animals and Experimental Design

Male Wistar rats of 16-19 weeks age, weighing 130-175g were procured from the Central Animal House, MNR college of Pharmacy, Sangareddy, Hyderabad, Telugana, India. The rats were kept in cages, 2 per cage, with 12:12 hr light and dark cycle at 25^0±2^0C. The rats were maintained on their respective diets and water *ad libitum.* Animal Ethical Committee's clearance was obtained for the study (MNR college of Pharmacy, Sangareddy, Hyderabad, Telugana CPCSEA/COP/07 dated 04-05-2017). Rats were divided into following 7 groups of 6 rats each:

Group I : Standard chow diet (Control)

Group II : High Fat Diet

Group III : High fat diet + Quercetin (15mg/kg B.wt)

Group IV : High fat diet + Gallocatechin (15mg/kg B.wt)

Group V : High fat diet + Luteolin (15mg/kg B.wt)

Group VI : High fat diet + Terminoside A (15mg/kg B.wt)

Group VII : High fat diet + Standard drug atorvastatin (1.2 mg/kg B.wt)

At the end of 63 days all the rats were sacrificed by cervical dislocation after overnight fasting. Just before sacrifice, blood was collected from the retro-orbital sinus plexus under mild ether anaesthesia and blood sample collected in heparinised 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.

Animal diet

The compositions of the two diets were as follows [20].

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%.

Biochemical estimation

Plasma samples were analysed for 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 *et al* (1972) method[21]. Ester cholesterol [22] and free cholesterol[22] 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* (1957)[23]. Extracts were used for the estimation of ester cholesterol and free cholesterol[24], triglycerides[25] and free fatty acids[26]

Statistical analysis

Results were expressed as mean ± SE of 6 rats in each group. The statistical significance between the groups were analyzed by using one way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Significance level was fixed at 0.05.

RESULTS

Table 1: List of compounds taken from *Terimala Arjuna* stem bark based on the availability of the structural details

S. No	Name	Phytochemicals	PDB & marwin Structures
1	Arjungenin	Triterpenoids	12444386
2	Arjunic acid	Triterpenoids	15385516
3	Arjunin	Triterpenoids	102316370
4	Arjunolic acid	Triterpenoids	73641
5	Terminic acid	Triterpenoids	12314613
6	Luteolin	Flavonoids and phenolics	5280637
7	Ethyl gallate	Flavonoids and phenolics	13250
8	Arjunone	Flavonoids and phenolics	14034821
9	Baicalein	Flavonoids and phenolics	5281605
10	Catechin	Flavonoids and phenolics	9064
11	Epicatechin	Flavonoids and phenolics	72276
12	Epigallocatechin	Flavonoids and phenolics	72277
13	Gallic acid	Flavonoids and phenolics	370
14	Gallocatechin	Flavonoids and phenolics	65084
15	Kempferol	Flavonoids and phenolics	5280863
16	Quercetin	Flavonoids and phenolics	5280343
17	Ellagic acid	Flavonoids and phenolics	5281855
18	Pyrocatechols	Tannins	289
19	Qudranoside VIII	Ursane triterpenoids	10675744
20	B-Sitosterol A	others	222284
21	Terminoltin	Triterpenoids	
22	2α,3β-dihydroyurs-12,18-oic acid 28-O-β-d-glucopyranosyl ester	Ursane triterpenoids	
23	2α,3β,23-trihydroxyurs-12,18-dien-28-oic acid 28-O-β-glucopyranosyl ester	Ursane triterpenoids	
24	Kajiichigoside F1	Ursane triterpenoids	
25	2α,3β,23-trihydroxyurs-23-trihydroxyurs-12,19-dien-28-oic acid 28-O-β-d-glucopyranosyl ester	Ursane triterpenoids	
26	Arjunolone	Glycosides	
27	Terminoside A	Glycosides	
28	Arjunaphthanoloside	Glycosides	
29	Olean-3β, 22β-diol-12-en-28 β-D-glucopyranosie-oic acid	Glycosides	
30	3-O-methyl-ellagic acid 4-O-β-d-xylopyranoside	Flavonoids and phenolics	

31	3-O-methyl ellagic acid 4′-O-α-l-rhamnophranoside	Flavonoids and phenolics	
32	Casurinin	Tannins	
33	Casuriin	Tannins	
34	Castalagin	Tannins	
35	Terflavin	Tannins	
36	Terchebulin	Tannins	
37	Punicalin	Tannins	
38	Punicalagin	Tannins	

Chemical structures of the above components were constructed by the MarvinSketch then the structures were minimized through Macromodel[27] using Merck Molecular Force Field (MMFF). The structure constructed using the MarvinSketch primarily given an unrefined molecular structure with bond angles and lengths distorted from their respective minima or with steric clashes between atoms, energy minimization process was used for correcting these flaws and to provide a stable confirmation

In order to investigate the binding capacity of a few important bioactive compounds of *Terminalia arjuna* stem bark with anti-dyslipidaemia activity, on HMG-CoA reductase enzyme protein in humans, we docked each compound to the target proteins. Computational docking studies of *T. arjuna* stem bark compounds with HMG-CoA reductase showed binding interaction with key residues. Docking analysis revealed the four (quercetin, gallocatechin, luteolin, terminoside A) compounds from *T. arjuna* has potent inhibitory action against HMG-CoA reductase (Table 2).

Table 2: Top scoring compounds with its interaction analysis, and with the length

S. No	Entry Name	Docking score	XP Score	Glide g - score	Glide energy	glide e - model	Interaction	H-Bond Length
1.	Quercetin	-8.31958	-8.32488	-8.32488	-42.8855	-58.4799	0-H…O=CGLY(560A) NH_2(GLY(560A)…O=C 0-H…O=C Gly (765B) 0-H…O=C Gly (765B)	2.114 2.416 2.381 2.146
2.	Gallocatechin	-7.56943	-7.57253	-7.57253	-47.1576	-58.2573	0-H…O=C Gly (765 B) 0-H…O=C Gly (765 B) 0-H…O-C Asp (767 B)	1.936 2.046 2.118
3.	Luteoline	-7.43479	-7.44749	-7.44749	-44.5225	-59.6608	0-H…O=C Gly (765 B) 0-H…O=C Gly (765 B) 0-H…O=C GLY(560A) NH_2(GLY(560A)…O=C	2.152 2.373 2.079 2.481
4.	Terminoside A.	-7.37531	-7.37851	-7.37851	-45.9534	-41.2107	O-H….O=C GLY(560A) O-H….0-HGLH (559A) O-H….O=CLEU (862A)	1.838 1.961 1.889

Evaluation of Hypolipidemic activity

The average body weight changes in control and phytoconstituents treated rats were presented in Table 3. The body weight of High fat fed treated rats (group II) were increased significantly ($p<0.001$) when compared with normal control group of rats (group I). The average body weight was reduced significantly ($p<0.001$) by the administration of quercetin and gallocatechin at the dose of 15mg/kg body weight as well as atorvastatin 1.2mg/kg b.wt when compared with HFD rats (group II).

Table 3: Average Body weight changes in control and phytoconstituents treated rats

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	133.76±0.97 ^{bNS}	191.62±1.21 ^{b*}	57.86±0.47 ^{b*}
Group II	133.50±1.01 ^{aNS}	256.52±1.68 ^{a}**	123.02±0.56 ^{a**}
Group III	144.3 ± 1.12 $^{aNS, bNS}$	210.36± 1.22 $^{aNS, b*}$	66.06 ± 0.36 $^{aNS, b*}$
Group IV	143.06±0.73 ^{aNS}, ^{bNS}	223.17±0.78 $^{aNS, b**}$	80.11±0.24 $^{aNS, b*}$
Group V	142.21±0.87 ^{aNS,bNS}	230.67±0.82 $^{aNS, b*}$	88.46 ± 0.45 $^{aNS, b*}$
Group VI	143.83±0.67 $^{aNS, bNS}$	238.75±0.77 $^{aNS, b**}$	94.92±0.28 $^{aNS, b**}$
Group VII	151.96±0.93 $^{aNS, bNS}$	219.19±0.78 $^{aNS, b**}$	67.23±0.34 $^{aNS, b**}$

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 and VII.

b → group II compared with groups III, IV, V, VI and VII.

Group I : Standard chow diet (Control)

Group II : High Fat Diet

Group III : High fat diet + Quercetin (15mg/kg B.wt)

Group IV : High fat diet + Gallocatechin (15mg/kg B.wt)

Group V : High fat diet + Luteolin (15mg/kg B.wt)

Group VI : High fat diet + Terminoside A (15mg/kg B.wt)

Group VII : High fat diet + Standard drug atorvastatin (1.2 mg/kg B.wt)

Effect of different phytoconstituents from *T. arjuna* stem bark on plasma lipid profile treated rats were presented in Table 4. There was a significant (p<0.001) increase in the level of plasma lipid profile in the group II rats fed with high fat diet when compared with the control (group I) rats. 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[28]. Administration of quercetin, gallocatechin, Luteolin and Terminoside A at the dose of 15mg/kg body weight to rat fed with HFD significantly (p<0.001) decreased in the level of plasma total cholesterol, free cholesterol, ester cholesterol, triglyceride phospholipids and free fatty acids as compared to HFD rats (group II). However, the treatment of quercetin and gallocatechin treated rats with HFD showed that the plasma lipid profile was restored to near normal as that of atorvastatin (group VII).

The Atherogenic Index (AI) is used as a marker to assess the susceptibility of atherogenesis. It was significantly (p<0.001) increased on feeding high fat diet to rats (group II) as compared to control rats (group I). Quercetin and gallocatechin treated rats significantly reduced the atherogenic index when compared with HFD fed rats (group II).

Effect of various phytoconstituents from *T. arjuna* stem bark on plasma lipoprotein in experimental rats were presented in Table 5. The plasma HDL-cholesterol levels were reduced in HFD rats (Group II) when compared to control group of rats (group I).After administration of quercetin and gallocatechin treated rats significantly (p<0.001) increased the beneficial HDL-cholesterol concentration in rats fed with high fat diet as compared to HFD rats (Group II). HFD fed rats (group II) are elevated levels of plasma Low Density Lipoprotein and Very Low Density Lipoprotein cholesterol when compared with the control rats (group I). High levels of LDL and VLDL-cholesterol are major risk factor for coronary heart disease [29]. After administration of quercetin, gallocatechin, Luteolin and Terminoside A at the dose of 15mg/kg body weight to rat fed with HFD showed significantly (p<0.001) reduced LDL-C, VLDL-C and increased the HDL-C, since HDL-C removes cholesterol to the liver for excretion, the increase in HDL-C will be appropriate for the reduced total cholesterol and thus reduce the risk of coronary artery disease[29].

Table 4: Effect of different phytoconstituents from *T. arjuna* stem bark on plasma lipid profile treated rats

Group	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Free fatty acid mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	Athrogenic index
Group I	110.55± 0.98^{b*}	25.30± 1.16^{b*}	85.19± 0.71^{b*}	42.80± 0.79^{b*}	96.48± 0.55^{b*}	83.61± 0.87^{b*}	1.68± 0.01^{b*}
Group II	183.50± 1.06^{a*}	59.22± 1.42^{a*}	124.28± 0.62^{a*}	59.35± 0.60^{a*}	142.42± 1.06^{a*}	157.95± 1.35^{a*}	5.53± 0.02^{a*}
Group III	103.77± 0.95$^{a**, b**}$	38.85± 1.93$^{a*, b*}$	64.91± 1.41$^{a**, b*}$	39.05± 0.57$^{a*, b**}$	103.38± 0.78$^{a*, b**}$	89.08± 0.57$^{a*, b**}$	1.63± 0.05$^{a**, b**}$
Group IV	107.81± 0.79$^{a*, b*}$	40.54± 0.59$^{a*, b*}$	67.43± 0.54$^{a*, b*}$	40,88± 0.47$^{a*, b*}$	109.02± 0.89$^{a*, b*}$	93.49± 0.58$^{a*, b*}$	1.64± 0.04$^{a*, b*}$
Group V	116.60± 0.80$^{a*, b*}$	44.98±. 0.96$^{a*, b*}$	71.62± 0.51$^{a*, b*}$	43.62± 0.55$^{a*, b**}$	113.61± 0.85$^{a*, b**}$	97.37± 0.29$^{a*, b**}$	1.65± 0.03$^{a*, b*}$
Group VI	122.01± 0.49$^{a*, b*}$	46.90± 0.48$^{a*, b*}$	75.13± 0.48$^{a*, b*}$	45.69± 0.38$^{a*, b*}$	120.68± 0.57$^{a*, b*}$	102.32± 0.61$^{a*, b*}$	1.65± 0.02$^{a*, b*}$
Group VII	98.68± 0.47$^{a*, b*}$	24.43± 0.92$^{a*, b*}$	74.24± 0.58$^{a*, b*}$	39.62± 0.43$^{a*, b*}$	93.63± 0.93$^{a*, b*}$	80.93± 0.81$^{a*, b*}$	1.32± 0.03$^{a*, b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : * < 0.001, ** < 0.05

Table 5: Effect of various Phytoconstituents from *T. Arjuna* stem bark on plasma lipoprotein in experimental rats

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)
Group I	65.27±0.57^{b*}	31.86±0.44^{b*}	16.72±0.17^{b*}
Group II	33.25±0.98^{a*}	110.10±0.95^{a*}	31.59±0.27^{a*}
Group III	59.02±0.83$^{a**, b*}$	25.19±0.37$^{a*, b*}$	17.81±0.11$^{a*, b*}$
Group IV	56.27±0.54$^{a*, b*}$	30.42±0.62$^{a*, b*}$	18.69±0.12$^{a*, b*}$
Group V	53.12±0.37$^{a*, b*}$	41.65±0.43$^{a*, b*}$	19.47±0.05$^{a*, b**}$
Group VI	53.08±0.30$^{a*, b*}$	44.07±0.49$^{a*, b*}$	20.46±0.12$^{a*, b*}$
Group VII	58.68±0.44$^{a*, b*}$	26.22±0.39$^{a*, b*}$	16.18±0.16$^{a*, b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : * < 0.001, ** < 0.05.

Effect of various phytoconstituents from *T. arjuna* stem bark on tissues ester and free cholesterol profile experimental rats were presented in Tables 6&7. The significant (P<0.001) increase in the levels of both free and ester cholesterol were observed in tissues of rats fed with high fat diet (group II) when compared to control rats (group I). This elevated cholesterol focus in blood dissemination may harm the endothelial cells coating the substantial supply routes and aorta and this may be an underlying occasion in the etiology of atherosclerosis[30]. Both tissue free and ester cholesterol reduced remarkably on quercetin, gallocatechin, Luteolin and Terminoside A at the dose of 15mg/kg body weight to rat fed with HFD than that of HFD treated group of rats.

Table 6: Effect of various phytoconstituents from *T. arjuna* stem bark on tissues ester cholesterol profile experimental rats

Groups	Ester cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	2.37 ± 0.02^{b*}	2.85± 0.02^{b*}	2.71±0.03^{b*}
Group II	3.80±0.03^{a*}	6.99±0.01^{a*}	6.87±0.034^{a*}
Group III	1.88±0.02$^{a*, b**}$	2.88±0.02$^{a*, b**}$	2.64±0.02$^{a*, b**}$
Group IV	2.00±0.02$^{a*, b*}$	2.93±0.15$^{a*, b**}$	2.78±0.02$^{a*, b*}$
Group V	2.17±0.01$^{a*, b*}$	3.12±0.01$^{a*, b**}$	3.08±0.01$^{a*, b**}$
Group VI	2.28±0.01$^{a*, b*}$	3.15±0.02$^{a*, b*}$	3.19±0.09$^{a*, b*}$
Group VII	1.88±0.01$^{a*, b*}$	2.70±0.02$^{a*, b*}$	2.66±0.04$^{a*, b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : * < 0.001, ** < 0.05.

Table 7: Effect of various phytoconstituents from *T. arjuna* stem bark on tissues free cholesterol profile experimental rats

Groups	Free cholesterol (mg/g tissue)		
	Liver	Heart	Aorta
Group I	0.82±0.01 ^{b*}	0.87± 0.06 ^{b*}	0.70±0.01^{b*}
Group II	1.68±0.01^{a**}	1.44±0.02 ^{a*}	2.49±0.06^{a*}
Group III	0.92±0.02^{a**,b**}	0.82±0.01 ^{a*,b**}	0.79±0.05^{a*,b**}
Group IV	0.93 ±0.05^{a*,b*}	0.87±0.04 ^{a*,b*}	0.81±0.06^{a*,b*}
Group V	1.05±0.08^{a*,b*}	0.88±0.02 ^{a*,b**}	0.83±0.07^{a*,b**}
Group VI	1.14±0.05^{a*,b*}	0.89±0.07 ^{a*,b*}	0.86±0.05^{a*,b*}
Group VII	0.84±0.01^{a*,b*}	0.78±0.01 ^{a*,b*}	0.78±0.04^{a*,b*}

Values are expressed as mean ± SE (n=6 rats), *P* values : * < 0.001, ** < 0.05.

Effect of various phytoconstituents from *T. arjuna* stem bark on tissues Triglyceride level experimental rats were depicted in Table 6. The concentrations of tissue triglyceride were elevated in rats fed high fat diet (group II) as compared to control rats (group I). HFD rats significant increase in the level of plasma triglyceride due to decrease in the activity of lipoprotein lipase [31]. Both plasma and tissue triglyceride levels were significantly reduced in rats treated with quercetin ,gallocatechin, Luteolin and Terminoside A at the dose of 15mg/kg body weight to rat fed with HFD in comparison with HFD rats (group II). The above phytoconstituents 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[32].

Table 8: Effect of various phytoconstituents from *T. arjuna* stem bark on tissues Triglyceride level experimental rats

Groups	Triglyceride (mg/g tissue)		
	Liver	Heart	Aorta
Group I	12.29±0.55^{b*}	14.32±0.68^{b*}	12.17±0.48^{b*}
Group II	28.76±0.81^{a*}	45.13±0.64^{a*}	31.25±0.39^{a*}
Group III	14.41 ± 0.59 ^{a*,b**}	17.57±0.50$^{a**, b**}$	15.07±0.27$^{a*, b**}$
Group IV	15.35±0.84$^{a*, b*}$	20.35± 0.64 $^{a*, b*}$	16.45±0.31$^{a*, b*}$
Group V	18.58±0.56$^{a*, b*}$	22.01± 0.62 $^{a**, b*}$	18.05±0.29$^{a*, b*}$
Group VI	20.33±0.80^{a*,b*}	23.39±0.33$^{a*, b*}$	20.19 ± 0.37 $^{a*, b*}$
Group VII	16.48±0.51^{a*,b*}	18.44±0.32$^{a*, b*}$	12.51 ± 0.46 $^{a*, b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values : * < 0.001, ** < 0.05.

Effects of various phytoconstituents from *T. arjuna* stem bark on tissues free fatty acids level experimental rats are presented in Table 9. An elevated level of tissues free fatty acids were observed in rat fed with HFD than control rats. It indicates the accumulation of lipid in tissues due to the increased consumption of saturated fats. Previous studies indicated that high fat diet containing saturated fatty acid altered lipid composition fluidity and permeability of membrane[33]. Treatment of Phytoconstituents of *T. arjuna* stem bark in HFD rats significantly decreased the tissues free fatty acid. Many herbal species active principles have been found to stimulate hepatic microsomal cytochrome P_{450} aryl hydroxylase activity, which is involved in the hydroxylation of endogenous steroid such as cholesterol[34].

Table 9: Effect of various phytoconstituents from *T. arjuna* stem bark on tissues Free fatty acids level experimental rats

Groups	Free fatty acids (mg/g tissue)		
	Liver	Heart	Aorta
Group I	11.16±0.31^{b*}	13.73 ±0.21^{b*}	11.57±0.33^{b*}
Group II	30.38±0.59^{a*}	46.13±0.42^{a*}	30.34± 0.66^{a*}
Group III	12.13± 0.21^{a**,b*}	15.30±0.36^{a**,b*}	13.08 ± 0.36$^{a*, b**}$
Group IV	12.35 ± 0.30^{a*,b*}	17.54±0.32 ^{a*,b*}	14.35± 0.43$^{a*, b*}$
Group V	13.23 ± 0.28^{a*,b**}	19.41±0.47 ^{a*,b*}	16.04± 0.31$^{a*, b*}$
Group VI	13.71±0.42^{a*,b*}	20.44±0.47^{a*,b*}	16.40± 0.61$^{a*, b*}$
Group VII	12.13±0.22^{a*,b*}	14.41±0.24^{a*,b*}	12.41± 0.32$^{a*, b*}$

Values are expressed as mean ± SE (n=6 rats), *P* values: * < 0.001, ** < 0.05.

CONCLUSION

The phytoconstituents from T. *arjuna* stem bark of quercetin, gallocatechin, Luteolin and Terminoside A were significantly reduced the plasma lipid and lipoprotein profile and reduced the atherogenic index. It also the significantly reduced the tissues free cholesterol, ester cholesterol, triglycerides and phospholipids when compared to standard drugs of atorvastatin. The findings therefore support the ethno medicinal uses of the various phytoconstituents of *T. arjuna* stem bark in the management of cardiovascular complications like atherosclerosis. Further, studies are required to again more insight in to the possible mechanism of action.

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