International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.3, pp 540-548, 2014-2015

Comparative Antihyperlipidemic Activity of Methanolic Extract of *Terminalia arjuna* Bark and its Phytosome

Sharma Shalini[1]*, Roy Ram Kumar[2], ShrivastavaBirendra[3]

***[1]Sunder Deep Pharmacy College, NH-24, Sunder Deep Nagar, Dasana, Ghaziabad, U.P., India**
[2]D.V. Institute of Pharmacy & Medicine, Karnal, Haryana, India
[3]School of Pharmaceutical Sciences, Jaipur National Universiy, Jaipur, Rajasthan, India

Abstract: Phytosomes are lipid soluble advanced herbal complexes known to increase the bioavailability of water soluble constituents. *Terminali aarjuna* is a plant rich in polyphenols. Methanolic extract of bark of *Terminalia arjuna* is well known for its lipid lowering effects. Phytosomes of methanolic extract of*Terminalia arjuna* were prepared.The antihyperlipidemic effect of orally administered methanolic extract of *Terminalia arjuna* bark and its phytosomeswas investigated on cholesterol fed albino wistar rats. At the end of the experimental period, the animals were killed and their plasma and tissue lipid components were estimated. Histological studies show that both methanolic extract of *Terminalia arjuna* bark and phytosomes of methanolic extract of *Terminalia arjuna* bark show negligible fatty infiltration and granular degeneration. The studies shows that complex was effective in decreasing the total cholesterol as well as triglycerides and raising high density cholesterol but the activity was low as compared to pure extract probably because of the presence of phospholipids.Thus phytosomal complexes of methanolic extract of *Terminaliaarjuna* should be prescribed to the patients accordingly.
Key Words: Arjuna Bark, Antihyperlipidemic activity, Phytosomes.

I. Introduction

Hyperlipidemia, the disorder of lipid metabolism is known to enhance the risk of coronary heart disease, fatty liver disease and carcinogenesis[1-3]. Abnormal levels or values of lipid profile such as total cholesterol, Low Density Lipoproteins (LDL), Very Low Density Lipoprotein (VLDL) and triglycerides are associated with atherosclerosis and ischemic heart disease. The serum LDL and VLDL are directly related to risk in both men and women while High Density Lipoproteins (HDL) is protective against atherosclerosis[4].

Terminalia arjuna Roxb (Family Combretaceae) is a large deciduous evergreen tree widely found throughout India. *Terminalia arjuna* (TA) is one of the most versatile medicinal plants with a wide spectrum of biological activity. TA has been known for its broad spectrum activities such as cardiac failure, hypocholsteremic, hypolipidemic, anticoagulant, antihypertensive, antithrombotic, dropsy, diuretics, antiinfective, antiasthamatic, hypolipidemic, antihyperlipidemic, treatment of rheumatoid arthritis and cancer[5-8].

TA has got promising hypolipidemic effects in respect to type-2 diabetic model rats [9].Ethanol and solvent ether fractions of TA is shown to exert lipid lowering activity in vivo in rats with induced hyperlipidemia and hamster fed a high fat diet[10]. Ethanolic fraction of TA apart from possessing antioxidant and hypolipidemic properties is also known to have therapeutic potential for the prevention of coronary arterial disease [11].

Phytosomes are advanced herbal products produced by binding individual components of herbal extracts to phosphatidylcholine resulting in a dosage form that is better absorbed and thus produces better results than the conventional herbal extracts[12]. Phosphatidylcholine is known to regulate the fluidity and permeability of the cell membrane. Phosphatidylcholine because of its lipophilic nature are known to cross cell membrane and thus increase the oral and topical absorption of herbal extract which are known to have poor bioavailability[13-15].

Phytoconstituents despite having excellent bioactivity in vitro demonstrate less or no in vivo actions due to their poor lipid solubility or improper molecular size or both resulting in poor absorption and poor bioavailability. Some phytoconstituents are destroyed in the gastric environment when taken orally[16].

With a view to enhance bioavailability of herbal drugs phytosomes have been prepared which binds individual components of herbal extracts to phosphatidylcholine resulting in a product that is better absorbed and thus produces better results than the conventional herbal extract[17]. In the present study the antihyperlipidemic effect of orally administered methanolic extract of *Terminalia arjuna* bark (METB) and phytosomes of methanolic extract of *Terminalia arjuna* bark (METBP) were investigated in albino wistar rats.

II. Material And Methods

All the reagents used were of analytical grade. Hydrogenated Phosphatidylcholine Phospholipon 90H was a gift sample from Lipoid, Germany. Phospholipid contains fatty acids (stearic acid and palmitic acid, 98%) and sum of the unsaturated fatty acids (oleic acid, linoleic acid, 2%). TA bark was purchased from a local supplier and authenticated by Dr. H. B. Singh, Chief Scientist and Head, Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR).

Extraction ofMethanolic Extract of *Terminalia arjuna* Bark (METB):

Coarsely powdered crude drug was placed in a stopper container with methanol and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until all the soluble matter has been dissolved. The mixture was then strained and the marc pressed. The combined liquids were clarified by filtration and dried to obtain METB.

Preparation of *Terminalia arjuna* Bark Extract Phytosome (METBP)

10 g Arjuna bark extract was dissolved in 200 ml of a mixture of 6 parts of methylene chloride and 1 part of methanol. 15 g of Hydrogented Phosphatidylcholine was added in small portion. When dissolution was complete, the solvent was distilled off under vacuum to small volume. The residue was diluted with 200 ml of methylene chloride, filtering any turbidity. Solvent was evaporated to small volume, the mixture was diluted with 300 ml of n-hexane and the product precipitated in form of light brown solid which was air dried. Product obtained was completely soluble in non-polar solvents[18].

IR Spectroscopic Studies of METB & METBP

An IR spectrum was recorded on SCHIMADZU 470 IR Spectrophotometer using KBr pellets in AIIMS, Delhi. Each sample and potassium bromide were mixed by an agate mortar and compressed into thin tablets. The IR obtained was elucidated for different chromatographic groups.

Antihyperlipidemic Studies

Animals

The Albino rats (Wistar strain) of either sex weighing 200 ± 30 gm bred in the animal house of Pinnacle Biomedical Research Institute (Reg. No. 1283/c/09/CPCSEA) Bhopal were used. All the animals were housed in polypropylene cages under controlled conditions of temperature (22±2°C), humidity (50±5%) and illumination (12 hrs light-dark cycle) with free access to food and water ad libitum. The animals were treated in accordance with the CPCSEA guidelines. The experimental protocol was approved by the Institute's Animal Ethical Committee with the approval Ref No. – PBRI/IAEC/11/PN-108.

Induction of hyperlipidemia

In the experimental rats, hyperlipidemia was induced by feeding them with cholesterol 500mg/kg and 1 ml HGNO (Hydrogenated Groundnut Oil) [19].

Experimental Design

The experimental animals were randomized into four groups (6 rats in each group). The experiment was conducted for a period of 28 days. The treatments of different group of animals were administered orally as described below.

Group A: Normal control rats were administrated 1ml of water orally from day 4 to 28 days.
Group B: Hyperlipidemic control rats were administrated 1ml of water orally from day 4 to 28 days [20].
Group C: Hyperlipidemic rats were administrated METB (250 mg/kg/day) orally from day 4 to 28 days [10].
Group D: Hyperlipidemic rats were administrated METBP (250 mg/kg/day) orally from day 4 to 28 days.

Collection of Blood Samples for Analysis

At the end of the 28 days animals were anaesthetized and blood was collected in EDTA coated eppendorf tubes. The blood was Centrifuge (Remi) for 10 minutes at 4ºC and plasma was separated which was used for the analysis of Total Cholesterol, Triglycerides, HDL, VLDL, and LDL by auto-analyzer (Star-21)

Histopathological Studies

At the end of the 28 days, food was withdrawn from the rats and they were fasted overnight but the animals had free access to water. They were then euthanized under chloroform vapour and sacrificed. Internal organs including pancreas and liver were surgically removed. Thereafter the tissues were suspended in 10% formal saline for fixation preparatory to histological processing. The fixed liver tissue was sectioned (5-micron thickness) and sections were firstly stained with basic dyes Heamatoxilin and Eosin (H&E) and photomicrographs were then developed[21].

III. Results

TA Sample was identified as *Terminalia arjuna* (Roxb. Ex DC.) Weight & Am.wide ref. no. NISCAIR/RHMD/Consult/-2011-12/1789/89. The obtained yield of METB was found to be 0.3%. METB was obtained as dark brown, shining crystals. The IR spectrum of methanolic extract of *Terminalia arjuna* showed absorption peaks at 3388 cm^{-1}, 3373 cm^{-1}, 1720.50 cm^{-1} and 1610.56 cm^{-1} in sample which indicate the presence of hydroxyl (Ar-O-H), carbonyl groups (carboxylic O-H) and aromatic carbon (Ar. C=C) respectively. The IR spectrum of methanolic extract of *Terminalia arjuna* confirmed the presence of OH at (3362.40 cm^{-1} broad), C-H stretching at (2926.01 cm^{-1}) as shown in Figure 1. IR spectrum of Phosphatidylcholine (Figure 2) at 1250cm-1 shows a band due to the group P=O but this band disappears in the spectrum of complex as shown in Figure 3. Flavonoid becomes bonded to the phospholipid via the polar end so as to strongly inhibit internal or intramolecular rotation. On the other hand the nonpolar portion of the lipid which is not involved in the formation of bonds is free to move so that the complex becomes strongly liposoluble.

Histological examination reveals that control group shown in Figure 4 is showing normal architecture. Hepatic lipid deposits appeared as small vacuoles within the cytoplasm of liver cells and showing fatty infiltration and granular degeneration in albino wistar rats fed the high fat diet i.e. cholesterol control group as shown in Figure 5. However the diets supplemented with the METB and METBP complex markedly reduced the number of these fatty droplets in rats and showed negligible fatty infiltration and granular degeneration as shown in Figure 6 & 7.

Administration of Cholesterol (500mg/kg) in albino wistar rats induces marked hyperlipidemia as evidenced by increase in the plasma levels of serum cholesterol as shown in table 2 as compared to normal group shown in table 1. Although the serum cholesterol level rose in all the groups after feeding either cholesterol alone or cholesterol with METB and METBP complex, the rise was much less in the rats treated with METB(Table3&4). Both METB and METBP were effective in antagonizing the rise of serum cholesterol caused by cholesterol feeding. Out of METB and METBP, the rise in serum cholesterol was lowest in METB. Experiments carried out also indicate that METB raises high density lipoproteins in rats(Table 5)[22]

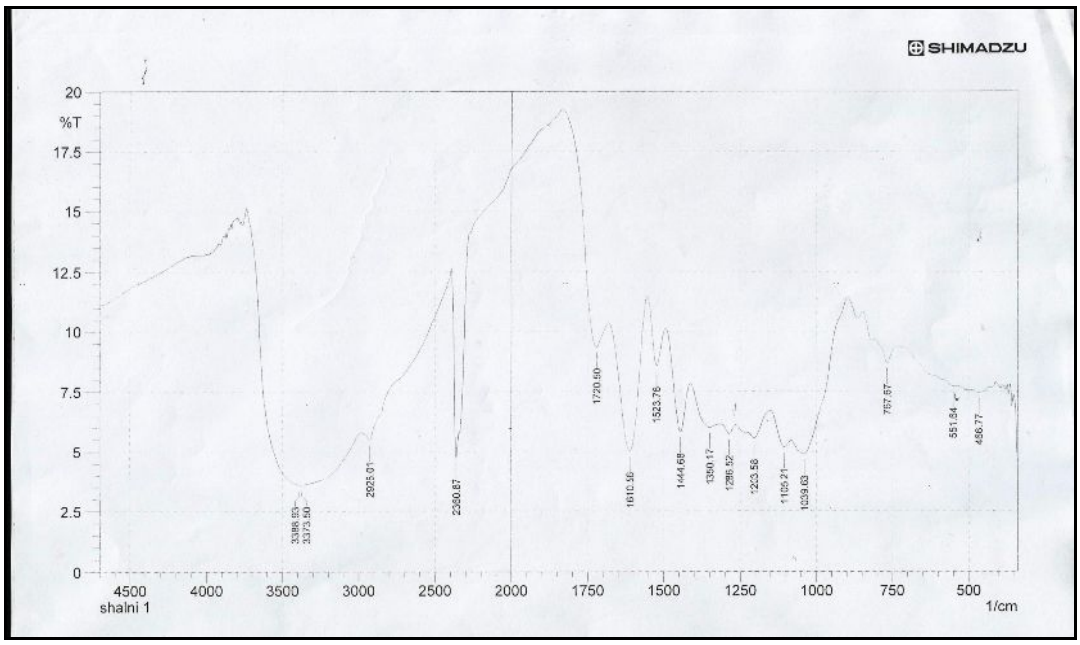

Figure 1: IR Spectrogram ofMETB

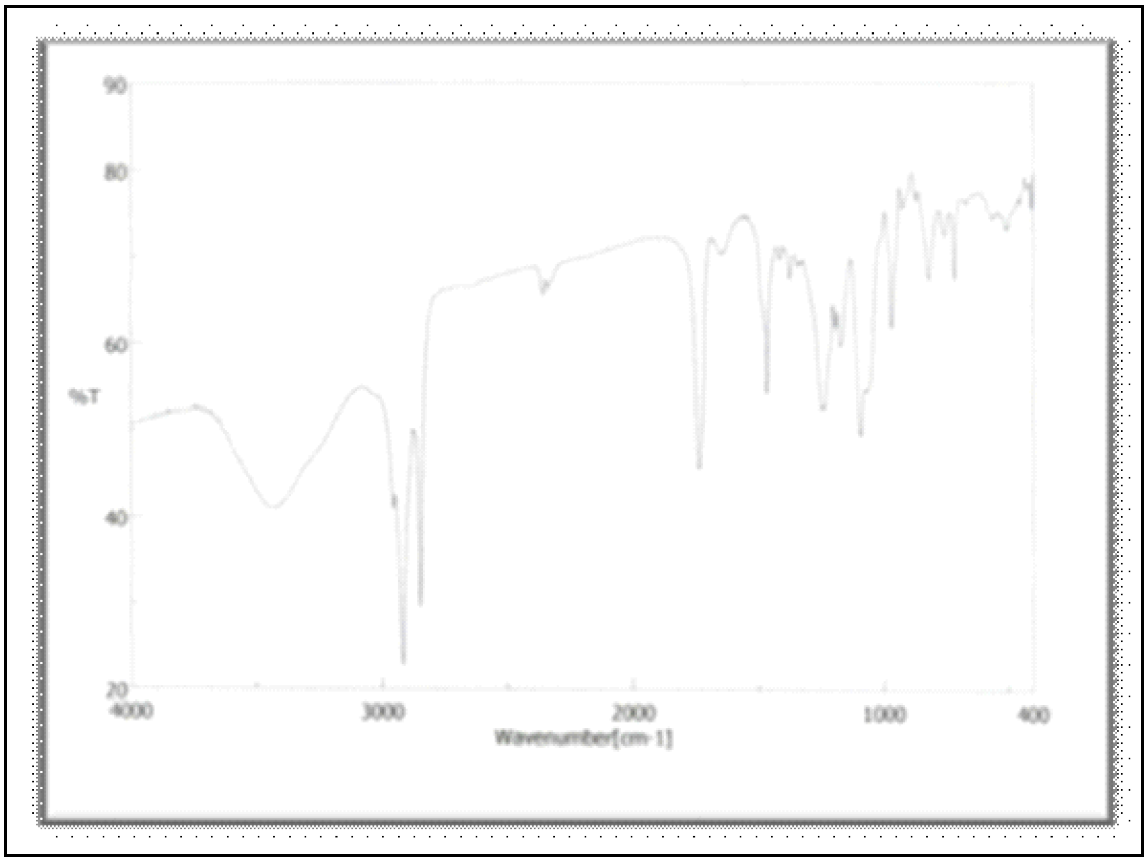

Fig 2: IR Spectrogram of Phospholipon 90H

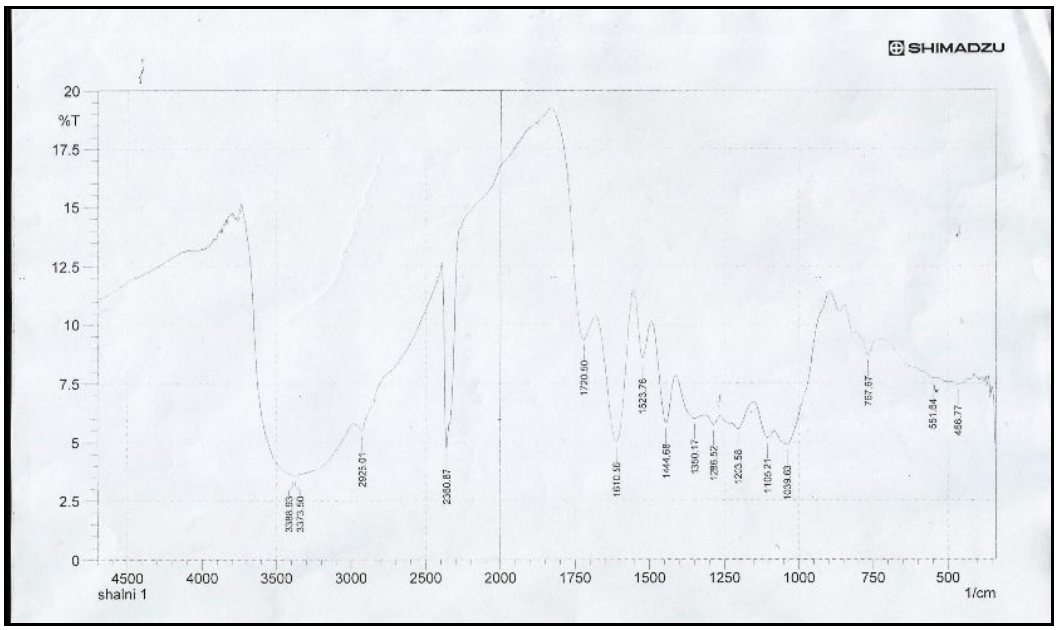

Figure 3: IR Spectrogram of METBP

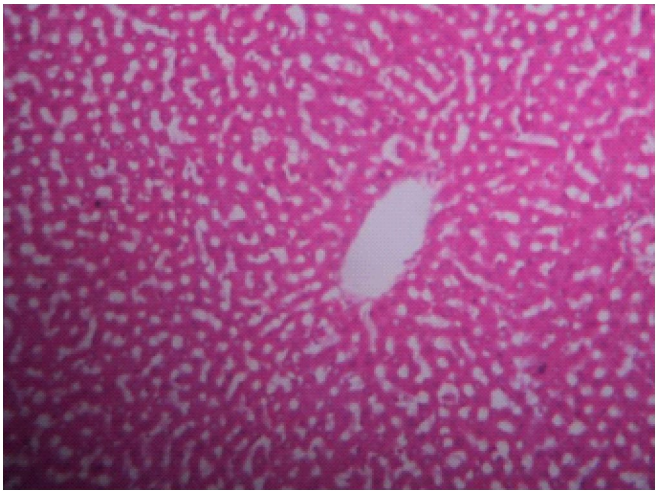

Figure 4: Histological Observations of Rats(Control Group)

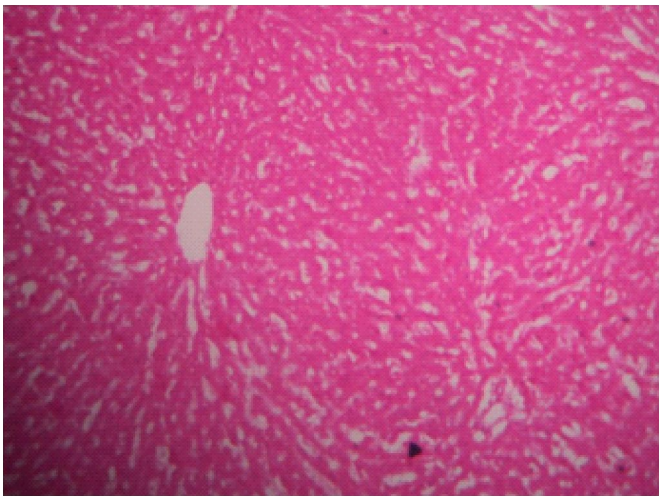

Figure 5: Histological studies of Rats: (Cholesterol Control)

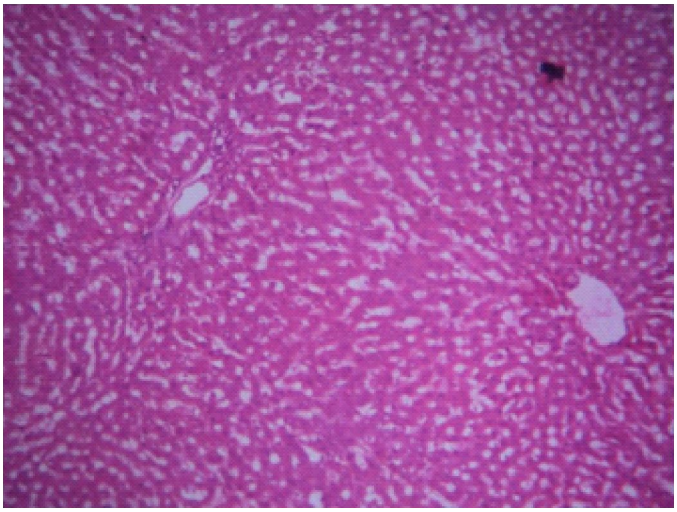

Figure 6: Histological studies of Group Treated with METB

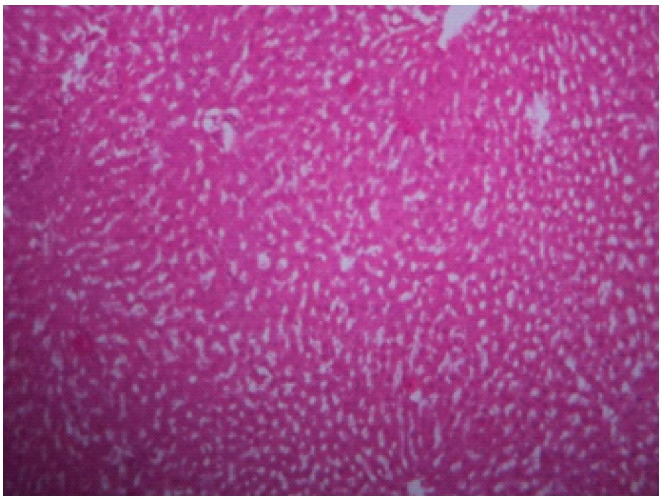

Figure 7: Histological studies of Group Treated with METBP

Table: 1-Lipid Profile of Group 1(Normal)

Animal	Total Cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL (mg/dl)		VLDL(mg/dl)		HDL(mg/dl)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	67.4	67.4	79.3	79.3	24.7	24.7	13.7	13.7	32.7	32.7
2	62.7	62.7	74.9	74.9	29.1	29.1	16.9	16.9	39.4	39.4
3	68.6	68.6	78.7	78.7	27.3	27.3	12.8	12.8	34.6	34.6
4	64.9	64.9	75.9	75.9	28.9	28.9	18.4	18.4	36.7	36.7
5	69.2	69.2	82.4	82.4	25.6	25.6	14.6	14.6	38.1	38.1
6	63.8	63.8	77.8	77.8	23.8	23.8	17.8	17.8	35.9	35.9
	66.1	**2.677312**	**78.16667**	**2.662079**	**26.56667**	**2.212389**	**15.7**	**2.313439**	**36.23333**	**2.408042**

Table: 2-Lipid Profile of Group 2 (Cholesterol Diet)

Animal	Total Cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL (mg/dl)		VLDL(mg/dl)		HDL(mg/dl)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	367.8	367.8	179.3	179.3	313.6	313.6	20.4	20.4	26.7	26.7
2	372.3	372.3	186.2	186.2	306.8	306.8	18.7	18.7	28.4	28.4
3	364.9	364.9	183.8	183.8	309.3	309.3	16.9	16.9	22.7	22.7
4	368.2	368.2	176.9	176.9	316.8	316.8	21.4	21.4	25.9	25.9
5	370.7	370.7	181.7	181.7	312.6	312.6	24.8	24.8	27.1	27.1
6	362.4	362.4	184.5	184.5	314.9	314.9	22.9	22.9	23.2	23.2
	367.7167	**3.642755**	**182.0667**	**3.478314**	**312.3333**	**3.688722**	**20.85**	**2.847279**	**25.66667**	**2.259794**

Table: 3-Lipid Profile of Group 3 (Cholesterol + 250 mg METB)

Animal	Total Cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL (mg/dl)		VLDL(mg/dl)		HDL(mg/dl)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	189.6	189.6	109.6	109.6	158.7	158.7	17.5	17.5	48.2	48.2
2	183.8	183.8	102.9	102.9	152.9	152.9	14.9	14.9	46.9	46.9
3	191.3	191.3	107.4	107.4	154.7	154.7	20.7	20.7	54.8	54.8
4	186.9	186.9	112.8	112.8	159.6	159.6	17.2	17.2	50.6	50.6
5	183.4	183.4	105.7	105.7	153.7	153.7	15.8	15.8	47.3	47.3
6	188.6	188.6	108.2	108.2	156.8	156.8	18.3	18.3	51.2	51.2
	187.2667	**3.180985**	**107.7667**	**3.375599**	**156.0667**	**2.736908**	**17.4**	**2.027807**	**49.83333**	**2.993771**

Table: 4-Lipid Profile of Group 4(Cholesterol + 250 mg METBP)

Animal	Total Cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL (mg/dl)		VLDL(mg/dl)		HDL(mg/dl)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	232.6	232.6	126.3	126.3	213.6	213.6	19.8	19.8	37.4	37.4
2	239.1	239.1	121.7	121.7	217.9	217.9	17.2	17.2	43.1	43.1
3	235.6	235.6	127.5	127.5	209.4	209.4	22.1	22.1	38.5	38.5
4	228.4	228.4	118.9	118.9	212.8	212.8	20.6	20.6	36.2	36.2
5	236.8	236.8	128.3	128.3	216.3	216.3	18.7	18.7	44.1	44.1
6	234.9	234.9	124.6	124.6	214.7	214.7	21.2	21.2	40.3	40.3
	234.5667	**3.704412**	**124.55**	**3.629738**	**214.1167**	**2.955278**	**19.93333**	**1.775012**	**39.93333**	**3.160169**

Table 5: Summary of Results of Antihyperlipidemic Activity in Albino Wistar Rats

Group no	Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
1	Control	66.1 ± 2.677	78.1 ± 2.662	26.5 ± 2.212	15.7 ± 2.313	36.2 ± 2.408
2	Cholesterol (500 mg/kg) + 1 ml HGNO	367.7 ± 3.642	182.0 ± 3.478	312.3 ± 3.688	20.8 ± 2.847	25.6 ± 2.259
3	Cholesterol (500 mg/kg) + 1 ml HGNO + METB (250 mg/kg)	187.2 ± 3.180	107.7 ± 3.375	156.0 ± 2.736	17.4 ± 2.027	49.8 ± 2.993
4	Cholesterol (500 mg/kg) + 1 ml HGNO + METBP (250mg/kg)	234.5 ± 3.704	124.5 ± 3.629	214.1 ± 2.955	19.9 ± 1.775	39.9 ± 3.160

IV. Discussion

The male albino wistar rats used in the study are reported as the ideal hypocholesterolemic models in previous studies[23]. Cholesterol induced hyperlipidemia is one of the animal model used for evaluation of antihyperlipidemic activity of drugs.

There may be several mechanisms responsible for antihyperlipidemic activity. Methanolic fraction is having multi targeted action due to the presence of beta sitosterol as well as flavones acting on the intestinal absorption of cholesterol and inhibiting HMG CoA reductase enzyme. Thus the hypocholesterolemic effect may be due to interference with the absorption of dietary cholesterol as well as bile acids from the intestine[24].

Increased stimulation of bile acid synthesis may lead to an increased utilization of cellular free cholesterol.[22] Thehypocholesterolemic activity might be mediated through increased cholesterol excretion in the feces. *Terminalia arjuna* increases fecal excretion of cholesterol and enhances the serum/plasma lecithin cholesterol acyl transferase (LCAT) activity in addition to accumulation of receptor mediated catabolism of LDL[25].

Tannins already reported by researchers may also be responsible for the lipid lowering effects[10,26]. The suppression of hepatic cholesterol biosynthesis might be the other possible mechanism responsible for antihyperlipidemic activity.

V. Conclusion:

Thus from the present study we conclude that METBP was effective in decreasing the total cholesterol as well as triglycerides and raising high density cholesterol but the activity was low as compared to METB probably because of the presence of phospholipids. Though the phytosome complexes are known to increase the absorption of the poorly bioavailable plant constituents, in this case results are contradictory as expected because of the presence of phospholipids. Thus phytosome complexes should be prescribed to patients accordingly.

VI. References

1. Anderson J.W., Hanna T.J., Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease,J.Nutrition 1999,129,1457S–1466S.
2. Festi D., Colecchia A., Sacco T., Bondi M., Roda E., Marchesini G., Hepatic steatosis in obese patients: clinical aspects and prognostic significance, Obesity Reviews2004,5,27–42.
3. Tseng T.H., Hsu J.D., Chu C.Y., Wang C.J., Promotion of colon carcinogenesis through increasing lipid peroxidation induced in rats by a high cholesterol diet, Cancer Letter 1996, 100,81–87.
4. Zafar A.M., Mohammad S., Saad B.Z.M. and Mahwish A.K., Herbal treatment for cardiovascular disease the evidence based therapy, Pak. J. Pharm. Sci.2010, 23(1),119-124.
5. Viswanatha G.L.S., Vaidya S.K., Ramesh C., Krishnadas N., Rangappa S., Antioxidant and antimutagenic activities of bark extract of *Terminalia arjuna*, Asian Pac.J. Trop. Med. 2010, 3, 965-70.
6. Kaur K., Arora S., Kumar S., Nagpal A, Antimutagenic activities of acetone and methanol fractions of Terminalia arjuna, Food Chem.Toxicol. 2002, 40, 1475-82.
7. Jain S., Yadav P.P., Gill V., Vasudeva N., Singla N, *Terminalia arjuna* a sacred medicinal plant: phytochemical and pharmacological profile, Phytochem. Rev. 2009, 8, 491-502.
8. Dwivedi S.&Jauhari R., Beneficial effects of Terminaliaarjuna in coronary artery disease,Indian Heart J.1997, 49, 507.
9. Morshed M.A., Haque A., Rokeya B., Ali L., Antihyperglycemic and lipid lowering effect of *Terminalia arjuna*bark extract on streptozotocin indiced type 2 diabetic model rats, Int. J. Pharm. Pharm.Sci 2011, 3(4), 450-454.
10. Chander R., Singh K., Khanna A.K., Kaul S.M., Puri A., Saxena R. et al.,Antidyslipidemic and Antioxidant Activities of different Fractions of *Terminalia arjuna* Stem Bark,Indian J.Clin.Biochem. 2004,19(2),141–148
11. Subramaniam S., Ramachandran S., Uthrapathi S., Gnamanickam V.R., Dubey G.P., Anti-hyperlipidemic and antioxidant potential of different fractions of *Terminalia arjuna* Roxb. bark against PX-407 induced hyperlipidemia, Ind. J. Exp. Bio. 2011; 49: 282-288.

12. Parris K., Kathleen H., A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybinphosphatidylcholine complex, Altern. Med. Rev. 2005, 10(3), 193-203.
13. Bombardelli E., Patri G.F, Complex compounds of bioflavonoids with phospholipids, their preparation and uses and pharmaceutical and cosmetic compositions containing them, US. Patent Number 5043323, 1991.
14. Bombardelli E., Curri S.B., Della L.R., Complexes between phospholipids and vegetal derivatives of biological interest,Fitoterapia 1989, 90 (l),1-9.
15. Gupta A., Ashawat M.S., Saraf S.,Phytosome: A Novel Approach towards Functional Cosmetics,J. Plant Sci.2007, 2(6), 644-649.
16. Kumar V.S., Kesari A., Herbosome-A novel carrier for herbal drug delivery, Int J Curr Pharm Res 2011, 3(3), 36-41.
17. Suresh R.N., Vinaya W.P., Vandana S.P., Neuropharmacological evaluation of Ginkgo bilobaphyto somesin rodents, Phytother. Res. 2006, 20(10), 901-905.
18. Bombardelli E., Patri G.F., Complex compounds of bioflavonoids with phospholipids, their preparation and uses and pharmaceutical and cosmetic compositions containing them, US. Patent Number 5043323, 1991.
19. Shaila H.P., Udupa S.L., Udupa A.L., Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis, Int. J. Cardio. 1998, 67(2), 119–124.
20. Reddy B.S., Kumar R., Bharavi K. and Ventates warlu U.,Hypolipidemic Acyivity of Methanolic Extract of *Terminalia arjuna* Leaves in Hyperlipidemic Rat Models, Res. J. Med. Sci. 2011, 5(3), 172-175.
21. Atangwho I.J., Patrick E.E., Godwin E.E., Mfon I.A. and Edem E.A., Histological Effect of Combined Extracts of VernoniaAmygdalinaand AzadirachtaIndicaon Normal and Diabetic Rats: the Pancreas and Liver, Res. J. Agri. Bio. Sci. 2010, 6(4), 514-521.
22. Shaila H.P., Udupa A.L.,Hypolipidemic effect of *Terminalia arjuna* in cholesterol fed rabbits, Fitoterapia 1997, 68, 405.
23. Mary N.K., Bahu B.H., Padikkala J., Antiatherogenic effects of Caps HT2 an herbal Ayurvedic medicine formulation,Phytomedicine 2003, 10, 474-482.
24. Srinivasan M.N., Srinivasan K.,Hypocholesteolemic efficacy of garlic smelling flower Adenocalymmaalliaceum in experimental rats, Indian J. Exp. Biol. 1995, 33, 64.
25. Shaila H.P., Udupa S.L., Nair N.S., Effect of Terminalia arjuna on experimental hyperlipidemia in rabbits,Int. J. Pharmacognosy 1997, 35, 1-4.
26. Nainwal P., Dhamija K., Tripathi S., Study of antihyperlipidemic effect on the juice of the fresh fruits of *Lagenaria siceraria*, Int. J. Pharm. Pharm. Sci. 2011, 3(1), 88-90.

*****