CHAPTER 6

DISCUSSION
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Phytochemistry:

Phytochemical analysis shows both the drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* does not have any toxic ingredients.

Gravimetry:

Increased body weight levels in hyperlipidemic diet clearly indicate lipid induced additional fat synthesis. The supplementation of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* shows protection against lipid induced lipogenesis.

Haematology:

Unchanged hematological profile in all the groups indicated both these two drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* are not having any adverse effect on hematopiosis. It also indicates *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* are safe to consume as per hematology concerned. Our findings are corporate with Arumugam Shivagurunathan et al1.

GENERAL

Lipid Profile:

Our result from lipid profile clearly indicates dyslipidemia in case of high lipid fed diets rats in group2, but surprisingly, both the drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* shown a protective action against lipid induced hyperlipidemia. Possibly, presence of high flavonoids and polythenol compounds with its antioxidant property act as hyperlipidemic agent2.

Feeding of high fat diet (group 2) may also result in excess hepatic triglycerides due to increased synthesis of triglycerides and increased de novo lipogenesis (DNL). The
supplementation of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* may induce an inhibitory effect on intestinal dietary fat absorption that leads to decrease triglyceride accumulation\(^3\). In addition, it may also possibly due to ability of greater functioning insulin to stimulate glucose transport mechanism in adipocyte and in skeletal muscle which is impaired due to hyperlipidemia resulting in insulin resistance. Impairment of insulin sensitivity or possibly insulin resistance leads to dyslipidemia\(^4\).

Hence, in our study the presence of beneficial phytoconstituents of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* inhibit effectively fat accumulation and ameliorate dyslipidemia in high lipid fed rats.

**Liver function test:**

Liver function tests clearly indicate no such changes of any parameters that is serum bilirubin, direct and indirect serum bilirubin, SGOT, SGPT, serum protein and alkaline phosphatase indicate the high lipid fat diet did not alter hepatocellular function in our study.

Both these two drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* cause no alteration in liver function test which indicates both these two drugs *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* are non toxic to Liver function tests and may be used as safe ingredients.

Usually most of the drugs based on pharmaceutical substances cause contraindication effects on hepatic function test. Hence major safety concerned any drug use in any research protocol is essential. Hence, both *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* may be considered as safe as per Liver function concerned\(^5\).
Serum electrolytes:

No change in levels of electrolytes in our study indicates neither hyperlipidemic diet nor *Terminalia Arjuna* (Arjuna) and *Embilica Officinalis* (Amla) any influences on electrolytes and its homeostasis.

Glucose regulation:

No change in Random Blood Sugar level in any groups in our study indicates unimpaired glucose homeostasis among all the groups, including the group of rats fed with hyperlipidemic diet.

SPECIAL

Histology of Atrium:

Normally, high fat diet causes remodeling the Atrial structure, but it is not confirmed whether high fat diet induces changes in histology of Atrial texture. In our study, we did not find any change in Atrial histological architecture in high fat diet fed rats.

Histological architecture of atrium of group 2 findings of high fat diet induced rats clearly indicate that short term, high fat diet feeding may not alter the Atrial wall architecture in our study.

Normally, excess lipid may stimulate mitochondria overload and activate myocardial molecular intimal cardiac remodeling.

Ventricle:

In our study on ventricular histology did not show any significant change of ventricular histology in group 2 rats, except we observed in few rats myocardium containing coronary artery showing an early changes of atherosclerosis which indicate minimal cardiac metabolic disturbances by high dietary fat.
Elastic artery:

We observe that in the endothelial layer of elastic artery shows an early change of atherosclerotic plaque and even we observe that there is a mild alteration in the arterial wall histology. These alterations may include arterial wall modification with component changes in the arterial wall and same in stiffer aorta. This study has been conducted on short term fat diet basis.

In our study early atherosclerotic changes in group 2 rats reflect less elasticity in arterial wall which may lead to increase in peripheral resistance and blood pressure⁶.

It has been already reported that elastic artery changes of tunica intima and tunica media increase with high fat diet and it leads to increase arterial stiffness from small arteries to large arteries⁷.

Increase thickness of elastic artery; tunica intima and tunica media are partly due to increase in smooth muscle cells. It was reported that aortic intima and media thickness was an earlier marker of preclinical atherosclerosis, which had been observed in group 2 in our study⁸.

The function of elastic fibers in the arterial wall is the maintenance of tension without constant expenditure of energy. According to Burton the arterial tension has a correlation to the amount of elastic tissue present in the vessel wall. Since coronary arteries arise from the root of aorta, they are subjected to maximum pressure during each cardiac cycle and hence have abundant elastic fibers to maintain arterial tension⁹.

From table no 8 and 9 we have clearly found decreased lumen, increased arterial wall thickness which again bring back to the normal in case of group 3, 4 and 5 with supplementation of drugs *Terminalia Arjuna* (*Arjuna*) and *Emblica Officinalis* (*Amla*).
The result indicates loss of arterial compliance with possible stiffening accompanied by histological modification of arterial wall due to high fat diet. Perhaps the internal elastic lamina or media component might be enriching fibers components such as collagen and elastin. The high fat diet induces changes in this vascular integrity and induces loss of elasticity. This increase in collagen was partly an addition to the bulk of the media but in later life it was partly at the expense of smooth muscle\textsuperscript{9} Thus, alteration of mechanical priority which may lead to severe cardiovascular dysfunction\textsuperscript{10}.

Although the mechanism is reinitiating arterial remodeling in high dietary lipid which induce metabolic disorder but earlier studies have reported that high lipid causes concomitant reduction in arterial luminal diameter\textsuperscript{11}.

In our study the supplementation of *Terminalia Arjuna (Arjuna)* and *Emblica Officinalis (Amla)* and both drugs groups, show a significant improvement in lumen diameter accompanied by a significant decrease of arterial wall thickness in rats fed with high lipid diet.

These results clearly show that improvement of elastic arterial property by treatment with *Terminalia Arjuna (Arjuna)* in group 3 and *Emblica Officinalis (Amla)* in group 4 and both the drug *Terminalia Arjuna (Arjuna)* group and *Emblica Officinalis (Amla)* treated group 5.

**Muscular Artery:**

High lipid fat diet did not show any alteration of a muscular artery of the table shows (Table No 10) at any level of tunica adventitia, tunica media or tunica intima. It clearly indicates the short term effect of high lipid diet apparently does not have any adverse impact on muscular arterial integrity.
Coronary artery:

Smoking, hypertension, diabetes, fibrinogen, and low density lipoprotein cholesterol (LDL cholesterol) are widely accepted coronary heart disease (CHD) risk factors. These risk factors are also associated with preclinical atherosclerosis, generally measured as the intima-media thickness (IMT)\textsuperscript{12}.

In our observation of coronary arterial wall and lumen integrity; changes have shown in the lumen area as an early change of atherosclerosis in tunica intima as well as tunica media in high fat fed rats.

Atherosclerosis is common condition which leads to inflammatory status of the micro vessels leading to the development of ischemic heart diseases or cerebrovascular disease or peripheral vascular disease. One of the common risk factor for atherosclerosis is high dietary lipid which induces pathophysiological vascular phenotypic alterations\textsuperscript{13}.

Atherosclerosis is a disease of the tunica intima. The tunica intimal layer is separated from the tunica media layer by the internal elastic lamina (membrane) (internal elastic lamina, elastica interna), which is a fenestrated sheet of elastic tissue\textsuperscript{14}.

The tunica media consists of multiple layers of smooth muscle cells and connective tissue (elastic fibers, collagen, proteoglycans). The amount of elastic tissue is less and the number of smooth muscle cells is greater in the epicardial coronary arteries than in other elastic vessels\textsuperscript{15}.

The tunica media consists of up to 40 layers of circumferentially or helically oriented smooth muscles. The normal tunica media ranges in thickness from 125-350 pm (average 200 pm)\textsuperscript{16}.

Tunica media thickness underlying diseased intima (atherosclerotic plaque) is considerably thinner, ranging from 16 to 190 pm (mean 80 pm)\textsuperscript{16}. The smooth muscle cells are embedded in a glycoprotein mix that stains heavily with the periodic acid-Schiff reactions (being PAS positive).
Collagen and elastic fibers are also present in this layer. The tunica media layer is separated from the tunica adventitial layer by the external elastic lamina (membrane) (external elastic lamina, elastica externa). The external elastic membrane is composed of interrupted layers of elastin and is considerably thinner than the internal elastic membrane\textsuperscript{14}.

The increase in tunica intima was found to be the basic pathological change which ultimately progress to atherosclerosis\textsuperscript{9}.

One of the most important initial events in the development of atherosclerosis is accumulation of cells containing excess lipid within the arterial wall which are mostly macrophages and transformed monocytes, those engulfs oxidized LDL to become foam cell of fat laden macrophages\textsuperscript{17}.

The tunica intima and greater part of the tunica media lack capillaries and receive nutrition by diffusion; hence oxygen and other nutrients including soluble blood lipids must diffuse from lumen through the intercellular substance of the intima and most of the media. With abnormal increase in intimal thickening this diffusion mechanism gets very much deranged resulting in insufficient oxygen tension in tissues of arterial wall\textsuperscript{9}.

Internal elastic lamina showed splitting, fraying, fragmentation and reduplication in various age groups\textsuperscript{9}.

Most of the drugs against atherosclerosis are basically the inhibitor of HMG CoA reductase which is important for regulating relimiting step for cholesterol biosynthesis. The substantially important of coronary arterial wall thickness and lumen diameter and lumen area after treatment with these two drugs \textit{Terminalia Arjuna} (Arjuna) and \textit{Embilica Officinalis} (Amla) might be due to their potential impact of HMG CoA reductase on pathways.
In our study increased coronary arterial wall thickness with concomitant reduction of the coronary arterial wall area (change of Anterioposterior and transverse diameter) also in group 2 rats attributed a possibility of high oxidized LDL, oxidative stress that results from ROS leads to transform monocytes to macrophage and further develop foam cell, which fills subintimal layer and form fatty streak in the coronary artery\(^{18}\).

The fat induced injury on subintimal layer may also initiate various cytokines and growth factors which stimulate migration and proliferation of smooth muscle cell that became intermix with the area of inflammation to form intermediary lesion. If these response continue further causes increase in thickness of coronary arterial wall with compensatory slow dilatation\(^{19}\).

It has been noticed that stimulation of proinflammatory markers, cytokines, chemokines activate atherosclerotic plaque. The antioxidant effect of *Terminalia Arjuna* (Arjuna) and *Embilica Officinalis* (*amla*) observed in present study of groups 3, 4 and 5 may be mediated by protecting LDL oxidation which is one of the early changes of atherosclerosis\(^{20}\).

Thickening of coronary arterial wall definitely compromise coronary arterial lumen diameter and surface area which we have noticed in our observation in case of high lipid fed rats. There is reduction in coronary arterial diameter, structural area with increase in wall thickness.

**Normalized wall index:**

It has been reported that normalized wall index indicator of cardiovascular diseases of and mean wall index might be useful to assess the atherosclerotic disease burden. Although in our study mean lumen area showed a significant change in group 2 as compared to normal. But many other cardiovascular diseases lumen area does not show any changes. Hence simply assessing lumen or area may be considered as less sensitive marker of the atherosclerotic disease burden than normalized wall index\(^{21}\).
Decrease in group 2 normalised wall index also indicate negative remodeling and in our study supplementation of drug *Terminalia Arjuna* (Arjuna) and *Emblica Officinalis* (Amla) both the drugs shows a remarkable improvement in normalized wall index which may be considered as passive indicator for coronary arterial structural integrity.
REFERENCES:


2. Saravanan Subramaniam et al. “Anti-Atherogenic Activity of Ethanolic Fraction of Terminalia Arjuna Bark on Hypercholesterolemic Rabbits” Evidence-Based Complementary and Alternative Medicine, Volume 2011, Article ID 487916, 8 pages.


