EFFECT OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* (AMLA) ON INTERMEDIARY METABOLISM OF ALBINO RATS FED WITH HYPERLIPIDEMIC DIET

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DECLARATION BY THE CANDIDATE

I hereby declare that this declaration/thesis entitled **"EFFECT OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* (AMLA) ON INTERMEDIARY METABOLISM OF ALBINO RATS FED WITH HYPERLIPIDEMIC DIET "** is a bonafide and genuine research work carried out by me under the guidance of **Prof Kusal K. Das,** Professor, Dept of Physiology, Karnataka, India. No part of this thesis has been formed the bases for the award of any degree or fellowship previously.

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ABBREVIATIONS

%	**- Percent**
µm	**-Micrometer**
µl	**-Micro liter**
mmol/L	**-Milimole/Liter**
µmol/L	**-Micromole/Liter**
nm	**-Nanometre**
ml	**-Millilitre**
gms	**-Grams**
gm	**-Gram**
g/dl	**-Grams/Desi litre**
g/L	**-Grams/Litre**
mg/kg	**-Milligram/Kilogram**
IP	**-Indian Pharmaceuticals**
b.wt	**- Body weight**
HR	**-Heart Rate**
IAEC	**- Institutional Animal Ethical Committee**
ICMR	**- Indian Council of Medical Research**
CPCSEA	**-Committee for the Purpose of Control and Supervision of Experiments on Animals (India)**
TLC	**-Thin Liquid Chromatography**
HPLC	**-High programmed liquid chromatography**
Hcl	**-Hydrochloride**
Conc	**-Concentration**
Na^+	**-Sodium**
K^+	**-Potassium**
Ca^{++}	**-Calcium**
Hb%	**-Haemoglobin Percentage**

RBC	**-Red Blood Corpuscles**
WBC	**-White Blood Corpuscles**
PCV	**-Packed Cell Volume**
MCHC	**-Mean Corpuscular Hemoglobin Concentration**
MCV	**-Mean corpuscular volume**
MCH	**- Mean Corpuscular Hemoglobin**
Neutro	**-Neutrophils**
Lympho	**-Lymphocytes**
Pt count	**-Platelet count**
Sr protein	**-serum protein**
Sr albumin	**-Serum Albumin**
TC	**-Total Cholesterol**
TGs	**-Trygleseroids**
TG	**-Trygleseroid**
LDL	**-Low Density Lipoprotein**
HDL	**-High Density Lipoprotein**
ILDL	**-Intermediate Low Density Lipoprotein**
IDL	**-Intermediate Density Lipoprotein**
IHD	**-Ischaemic Heart Disease**
FFA	**-Free Fatty Acids**
VLDL	**-Very Low Density Lipoprotein**
ApoB	**- *Apolipoprotein B***
Ox LDL	**- oxidised low density lipoproteins**
OxLDLS	**- Oxidatively Modified Low Density Lipoprotein.**
MCP1	**- Monocyte Chemotactic Protein-1**
NOx	**-NITRIC OXIDE**
TNF Alpha	**- Tumor Necrosis Factor Alpha**
MIP1 Alpha	**- Macrophage Inflammatory Protein 1-alpha**

VCAM1	**- Vascular cell adhesion molecule-1**
ICAM1	**-Intercellular Adhesion Molecule 1**
SMCs	**-Smooth Muscle Cells**
ROS	**-Reactive Oxygen Spaces**
HMG CoA	**- Hydroxy Methyl Glutaryl-*CoA* Reductase**
H_2O	**-Water**
H_2O_2	**-Hydrogen Peroxide**
O_2	**-Oxygen**
ATP	**-Adenosine Tri Phosphate**
ADP	**-Adinosine Di Phosphate**
RPM	**-Rotation Per Minute**
SGOT	**-Serum Glutamic Oxaloacetic Transaminase,**
SGPT	**-Serum Glutamic Pyruvic Transaminase,**
ALP	**-Alkaline Phosphatase Level**
ALT	**-Alanine Transaminase**
AGAPPE	
LDH	**- Lactate Dehydrogenase**
NAD	**- Nicotinamide Adenine Dinucleotide.**
NADH	
mRNA	**- messenger Ribonucleic acid**
SOD	**- Superoxide dismutase**
GSH	**-Reduced glutathione**
NOS3/eNOS	**-Endothelial nitric oxide**
iNOS	**-Induced nitric oxide**
OGTT	**-Oral glucose tolerance test**
LFT	**-Liver function test**
KFT	**-Kidney function test**
HRV	**-Heart rate variability**
LF/HF	**-Low frequency/ High frequency**

VLF	**-Very low frequency**
ECG	**-Electrocardiogram**
MDA	**-Malondialdehyde**
FBS	**-Fasting blood sugar**
EEO	**-Ethanolic extract of *Emblica Officinalis***
DM	**-Diabetes mellitus**
SFA	**-Saturated fatty acids**
MUFA	**-Monounsaturated fatty acids**
PUFA	**-Polyunsaturated fatty acids**
TFA	**-Trans fatty acids**
HPL	**-Hormone sensitivity lipase**
ACAT	**-Acetyl CoA acetyltransferase**
Acyl-CoA	**-Cholesterol acyltransferase**
CVD	**-Cardiovascular diseases**
ATM	**-Adipose tissue microphages**
CaM	**-Calmodulin**
DNA	**-Deoxyribonucleic acid**
ANS	**-Autonomic function system**
FFT	**-Fast fourier transform**
AR	**-Analytical grade reagents**
EDTA	**-Ethylene diamine tetra acetic acid**
LCAT	**-Lacithin Chelosterol acyl transferees**
L	**-Length reaction**
vt	**-Total volume**
vs	**-Sample volume**
Rt	**-Right**
Lt	**-Left**
RAAS	**-Renin –angiotensin-aldesterone system**
NAFLD	**-Non alcoholic fatty liver disease**

H&E	**- Hematoxylin and Eosin**
TCA	**-Tri chloro acitic acid**
TBA	**-Thiobarbutic acid**
DNPH	**Dinitrophenyl hydrazine**
DNTB	**-Dithiobis-2-nitrobenzoic acid**
FeCl3	**-Ferric chloride**
HNF Alfa	**-Hepatocyte nuclear factor**

ABSTRACT

Background:

High fat diet alters lipid profile, glucose homeostasis and possibly induce sympatho-vagal imbalance with oxidative stress. Modern drugs like Statin group are routinely used to combat patients with dyslipidemia have shown some contraindications. Hence more focus has been put forward on using natural products to treat against dyslipidemia and its associated complications. *Emblica Officinalis* belongs to Euphorbiacae family possess many active phytochemical components like gallic acid, tannin, flavonoids, emblicanin A and B and ellagic acid. Tannins and flavonoids present in *Emblica officinalis* were documented as glucose lowering, lipid lowering agents.

Aims & Objective:

The present study was aimed to assess ethanolic extract of *Emblica officinalis* (EEO) on high dietary fat induced alterations of lipid metabolism and cardiovascular system in experimental animals.

Materials & Methods:

Ethanolic extract of *Emblica Officinalis* was prepared and phytochemical analysis was done. Rats were divided into five groups, having six rats in each group as following; group 1-control, group 2 (+ EEO 100 mg/kg/b. wt); group 3 (fed with high fat diet); group 4 (fed with high fat diet+ EEO100mg/kg/b. wt) and group 5 (fed with high fat diet + atorvastatin 4mg/kg/ b. wt). The treatments were continued for 21 days. Gravimetric parameters and electrophysiological parameters of cardiac autonomic functions were recorded. Further evaluation of lipid profile, glucose homeostasis, oxidative stress was also done.

Results:

Significant alteration in serum lipid profile, glucose homeostasis, cardiac autonomic functions and oxidative stress were observed in group 3 rats (30% fat). Supplementation of EEO improved glucose homeostasis, cardiac autonomic functions and altered lipid profiles in rats fed with high fat diet (group 3). Histopathological observations also revealed morphological changes in elastic artery and liver in group 3 rats (30% fat). But supplementation with EEO remarkably improved histological architecture of elastic artery and liver in rats fed with high fat diet (group 3)

Conclusion:

Study indicates the protective role of EEO against high dietary fat induced dyslipidemia, cardiac autonomic malfunctions and altered glucose homeostasis in rats.

Key Wards: *Emblica Officinalis*, High fat diet, Lipid profile, sympatho-vagal balance, glucose homeostasis.

CHAPTER 1

INTRODUCTION

INTRODUCTION:

Intermediary metabolism plays very vital role in maintaining homeostasis between physiological functions of the body. The body's intermediary metabolism encompasses all the chemical process involved in energy production, energy release & growth. The goal of intermediary metabolism is to maintain a constant & adequate supply of fuel for all the organs of the body. It is because energy acquisition by the body is intermittent whereas energy expenditure is continuous. So body needs to store & then parcel out energy in a carefully coordinated fashion. This homeostasis is maintained by major organs like liver, brain, muscles and adipose tissues[1].

In recent years, life styles are over influenced by an excess consumption of high fat diets and greater than 83% of high fraction of saturated fatty acid with less amount of mono and poly unsaturated fatty acid impair metabolic function in great extent[2]. Overconsumption of high fat diet may increase a positive energy balance and develops overweight and obesity states[3]. Subsequently long term excess consumption of high fat diet causes hyperlipidemia, type 2DM, altered sympatho-vagal balance, insulin resistance and fatty liver diseases[4]. Hyperlipidemia occurs due to high levels of lipids especially total cholesterol, triglycerides and insufficient HDL which ultimately causing atherosclerosis and diseases associated with it[5].

Diabetes and hyperlipidemia are two main factors which revolve around the pathophysiological effects of abnormal lipid levels and insulin resistance[6]. Excess FFA storage from abnormal lipid metabolism leads to insulin resistance in peripheral cells, eventually causing hyperinsulinemia, hyperglycemia along with hyperlipidemia. However diabetes mellitus develops through pre diabetic state and land up in diabetic state if dietary lipid is not well regulated[4]. Hence retaining blood glucose homeostasis efficiently, has become indispensible concern in prevention of diabetes mellitus.

The new millennium has evidenced the emergence of a modern epidemic; metabolic syndrome with revolting consequences to the health of human worldwide[7]. To reduce the risk associated with high lipid levels, many hypolipidemic drugs and therapies have been developed. Modern drugs like Statin are popularly used to treat hyperlipidemea. Statins are fundamentally acted as HMG-CoA enzyme inhibitor[8]. Statins effectively act on increased levels of LDL but have shown some contraindications. For this reason relatively safe therapeutic alternative to fight against dyslipidemia including its associated complications is need of the hour. Nowadays there is increased interest towards using herbal drugs and therapies from natural sources against hyperlipidemea and its associated diseases. Hence, for treatment of hyperlipidemea with its associated diseases, much attention has been focused on the use of natural products which have very few side effects[9].

Emblica Officinalis (Amla) holds the prime place in the context of such medicinal properties like hypolipidemic, hypoglycemic and multimode cardio protective activities. *Emblica officinalis* belongs to Euphorbiacae family, commonly known as Amla or Indian gooseberry. It has been reported that *Emblica officinalis* contains many biological active components like polyphenols, tannins, gallic acids, emblicanin A & B and flavonoids[10]. Amla have shown many beneficial effects in variety of diseases like diabetes, eye disorder, scurvy, aging and rheumatism. Beside these affects most of the time amla is used as a cardiac tonic[11]. It is a strong antioxidant and found to have some influences on regulation of lipid metabolism and glucose metabolism[12].

REFRENCES:

1. Gerald I. Chapter 58 Metabolism. In Walter F. Boron, Emile L. Boulpeap, Medical Physiology 2nd edition Sounders Elsevier; 2009:1213.
2. Medai E, Lima Leopoldo AP, Pererira Junior PP, Leopold AS, Campos D HS, Raimundo JM,et al. Could high fat diet rich in unsaturated fatty acids impair the cardiovascular system?. Can J Cardiol. December 2010;26(10): 542-48.
3. Ding S, Fan Y, Zhao N, Yang H, Ye X, He D, et al. High fat aggravates glucose homeostasis by chronic exposure to bisphenol A. J. Endocrinol 2014;221(1):167-79.
4. Chang WC, Wu JS, Chen CW, Kuo PL, Chien HM, Wang YT, et al. Protective effect of Vanillic acid against hyperinsulinemia, hyperglycemia and hyperlipidemia via alleviating hepatic insulin resistance and inflammation in high fat diet(HFD) fed rats. Nutrients 2015;7(12):9946-59.
5. Shaik RP. Evaluation of hypolipidemic activity of methanolic extract of Ficus Dalhousiaemiq on triton-X and high fat diet indiced hyperlipidemia in albino rats. EJPMR 2014;1(1):183-91.
6. David S, Paul Z. Diabetes and hyperlipidemia: A direct quantitative analysis- A direct analysis of the effects of insulin resistance on lipid levels in relation to atherosclerosis coronary artery disease. WJCD 2012; 2:20-25.
7. Basciano H, Federico L, Adeli K. Fructose, insulin resistance and metabolic dyslipidemia. Nutrition & Metabolism 2005;2:5
8. Lankin VZ, Tikhaze AK, Kukharchuk VV et al. Antioxidents decreases the intensification of low density lipoprotein in vivo Peroxidation during therapy with statins. Molecular Cell Biochemistry 2003; 249(1-2):129-40.
9. Cooke JP. Nutriceuticals for cardiovascular health. American Journal of Cardiology 1998; 82(9).

10. Patel SS, Goyal RK, Shah RS, Tirgar PR, Jadav PD. Experimental study on effect of hydroalcoholic extract of *Emblica Officinalis* fruits on glucose homeostasis and metabolic parameters. Ayu 2013; 34(4):440-44.
11. Varghese LS, Alex N, Ninan MA, Soman S, Jacob S. evaluation of in vitro antibacterial activity whole plant (fruits, seeds, stem, leaves and roots) of *Emblica Officinalis* gaertn. International journal Ayurvedic & Herbal Medicine 2013; 3(6):1420-25.
12. Antony B, Merina B, Sheeba V, Mukkadan J. Effcet of standardized Amla extract on atherosclerosis and dyslipidemia. Indian journal of pharmaceutical Sciences 2006; 68(4):437-41.

CHAPTER 2

REVIEW OF LITERATURE

2.1: Lipids and dietary fatty acids:

The term LIPID in general defined as any molecule which is insoluble in water and soluble in organic solvents[1]. Lipid has broad range of molecules. Mainly lipids are divided into eight different categories i.e.fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides. The prime biological functions of lipids are to store energy in the form Triglycerols, as a structural molecule in the form of phospholipids in cell membrane and as a signaling molecule[2]Fats are a part of lipids composed of fatty acids in their esterified form i.e acylglycerols[1]. Fat is the dietary nutrient with the greatest energy density as it supplies 9 cal per gram. Dietary fats are mainly concerned with one of the eight categories i e fatty acyles which mainly includes fatty acids. Fatty acids are building blocks of lipids[3]. Fats in the diet are mainly composed of triglyceride, a molecule made of three fatty acids and a glycerol as a backbone. Fatty acids are of different types such as first, they are characterized by the number of double bonds. Saturated fatty acids (SFA) have no double bonds, while monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. These double bonds have either the cis or trans configuration. Most unsaturated fatty acids in the diet have the cis configuration, but trans-fatty acids (TFA) are also present. Second, the position of the double bond varies. Third, fatty acids differ in chain length, though the number of carbon atoms is usually an even number. Existing evidence says that the intake of fatty acids is an important determinant of the serum lipid and lipoprotein profile[4].

2.2: Fatty acids metabolism:

Beta oxidation is the mitochondrial process in which there is stepwise release of conserved energy of the fatty acids. Fatty acid oxidation occurs in two stages first in the cytoplasm then in the mitochondria. Fatty acids are long carbon chain molecules which lose 2

carbon molecules in each step to generate acetyl –CoA as an intermediate. Fatty acid oxidation also results in the reduction of NAD^+ and FAD^+ cofactors to generate NADH and $FADH_2$ [2].

In the cytoplasm, firstly Fatty acids get activated to acyl CoA through fatty acyl CoA synthase. Then acyl CoA is comlexed with carnitine by acyl carnitine palmitoyltransferase1 for transfer into the mitochondrial membrane.

In the mitochondrial stage carnitine is subsequently removed from the acyl CoA via acyl carnitine transferase 2. Further acyl CoA is degraded until long chain fatty acid becomes completely into acetyl CoA[1].

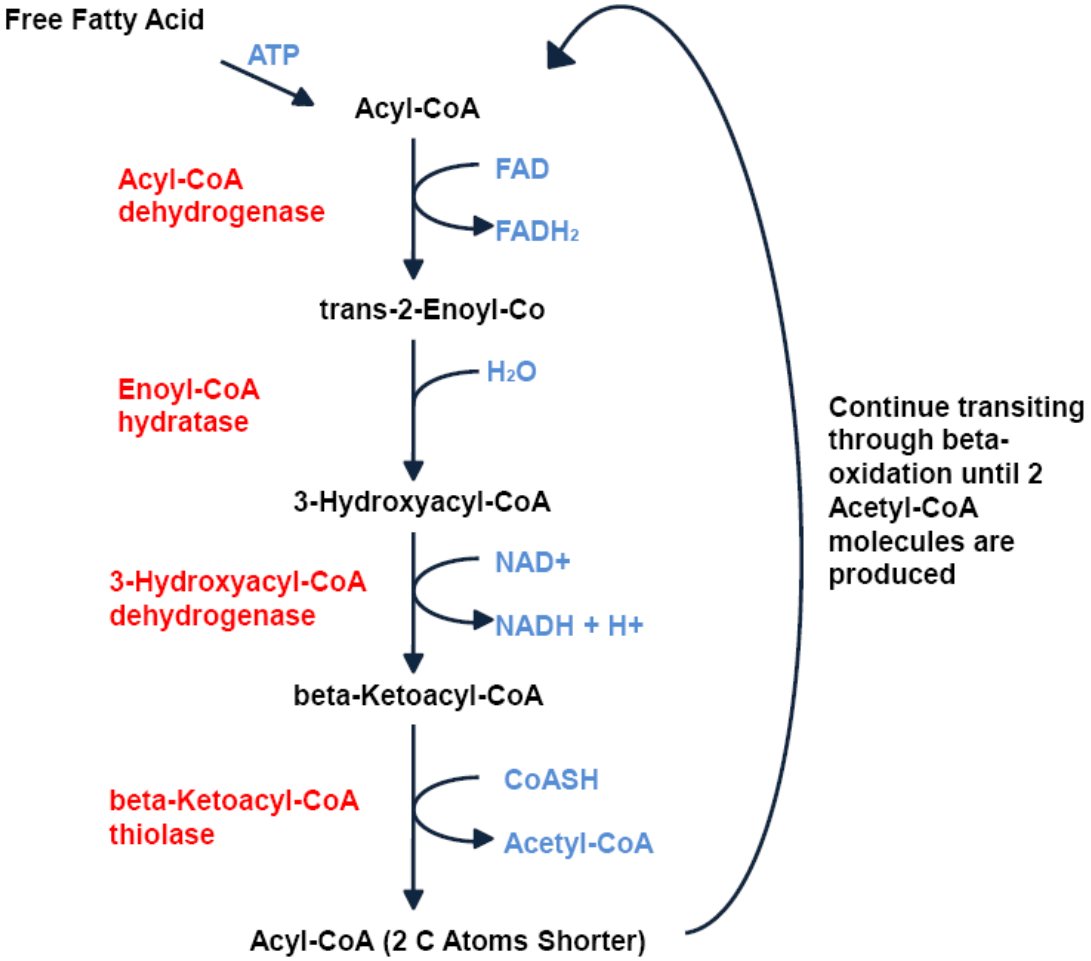


Figure: 2.1: ß Oxidation pathway

2.3: De Novo synthesis of fatty acids (Lipogenesis)

When there is increased concentration of acetyl CoA in the cytoplasm occurs, then fatty acid synthesis pathways get activated. Acetyle CoA is the major precursor and source for the carbon atoms. NADPH is used as a reluctant and ATP provides energy for fatty acid biosynthesis. Excess amount of carbohydrate and protein obtained from the diet can be converted to fatty acids which are stored as triacylglycerols. Mainly synthesis of fatty acids occurs in the liver and lactating mammary glands and to the lesser extent in adipose tissue, kidney and brain[1].

Adipocytes are store fat as energy in the form of triglycerides (TGs) and release them according to the need of individual. Hence these TGs must be separated first into their parts i.e one glycerol molecule and three free fatty acids by the lipoprotein lipase (LPL), which is released by the adipocyte. Glycerol stays in the blood whereas free fatty acids are transported into the adipocyte. Later on these are reformed in TGs and stored in lipid droplets. Their release is activated by the hormone sensitive lipase (HPL) and after their breakdown; free fatty acids are transported into the blood stream[5].

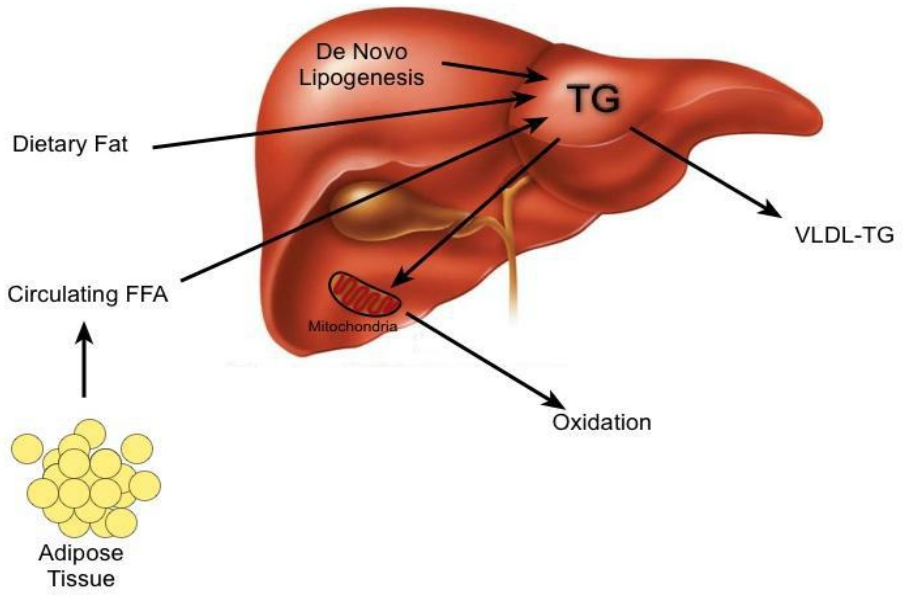


Figure 2.2: De Novo synthesis of fatty acids (Lipogenesis)

2.4: Gene regulation on fat:

Dietary fat is an important macronutrient for the growth and development of all organisms. Along with this its role as an energy source and its effects on membrane lipid composition, dietary fat has profound effects on gene expression, leading to changes in metabolism, growth, and cell differentiation. Specific fatty acid–regulated transcription factors include peroxisome proliferator–activated receptors (PPARα, -β, and -γ), HNF4α, NFκB, and SREBP1c. These factors are regulated by (*a*) direct binding of fatty acids, fatty acyl–coenzyme A, or oxidized fatty acids; (*b*) oxidized fatty acid (eicosanoid) regulation of G-protein–linked cell surface receptors and activation of signaling cascades targeting the nucleus; or (*c*) oxidized fatty acid regulation of intracellular calcium levels, which affect cell signaling cascades targeting the nucleus[6].

Adipocytes secrete number of proteins like leptin, adiponectin, TNF α, IL-6.

a) Leptin: is basically polypeptide of 17-kDa containing 167 amino acids and its secretion is directly related to adipose tissue mass and type of food ingested. It major function is to regulate food intake and energy balance via hypothalamic pathways. Leptin levels decrease with respect to caloric restriction and weight loss. It is a product of the *ob* gene, is a hormone derived from adipocyte in proportion to the degree of obesity[6].

b) adiponectin: it is also polypeptide of 30-kDa. Its biological effects depend on the relative circulating concentrations of its isoforms and the tissue specific expression of its receptor subtypes. Its levels decrease before the onset of obesity and insulin resistance whereas they are increased with improved insulin sensitivity[7].

c) TNF α,: it is a 26-kDa transmembrane protein. It is a proinflammatory cytokine involved in a various physiological processes like inflammation, proliferation and apoptosis. In obese

individuals and in animal models of obesity, TNFα expression is increased in abdominal fat and skeletal muscle[7].

d) Interleukin 6(IL-6): a pro-inflammatory cytokine that is expressed in and released by adipose tissue and whose circulating levels increase with obesity[8].

2.5: Major lipids in the body:

Major forms of dietary fats are Triglycerols (94%), cholesterols (1%) and phospholipids (5%).

1. Triglycerols: Body stores energy in the form of triglycerols. They are insoluble in water and because of their non polar nature they are popularly known as "Neutral fats". They are mainly synthesized in liver and adipose tissue.

2. Cholestrols: it is an integral component of cell membrane and influences membrane permeability. It is a sterol mainly found in animals. it is also major component of apolipiprotein. High levels of cholesterols are a potential risk factor for cardiovascular disease.

3. Phospolipids: phospholipids are structural components of biological membranes. It is composed of two glyceride molecules such as diglycerides[9].

2.6: Lipoproteins:

Fat absorbed from the diet and fat synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. The lipoprotein system evolves in transporting fats in the aqueous environment of the plasma[10].

Five main types of lipoproteins are

a) Chylomicrons

b) Very low density lipoprotein (VLDL)

c) Intermediate density lipoprotein (ILDL)

d) Low density lipoprotein (LDL)

e) High density lipoprotein (HDL).

f) Lipoprotein

Triglycerols and cholesterols are circulated by lipoprotein particles. Usually these particles are having hydrophobic lipid core consisting of triacylglycerols and cholesterol esters and hydrophilic surface is made of phospholipids and free cholesterol. The larger triacylglycerols-rich chylomicrons and very low density lipoproteins supply triacylglycerols to tissues. Low density lipoproteins (LDL) and high density lipoproteins (HDL) are mainly maintaining the regulation of the cellular cholesterol content. LDL particles transfer cholesterol to the cells and HDL particles remove cholesterol and transport it to the liver for reverse transport[11]. The cellular regulation of cholesterol biosynthesis is mainly controlled by the regulation of HMG-CoA reductase enzyme. Cholesterol levels control this enzyme by itself as a feedback inhibitor for existing HMG-CoA reductase. When cholesterol level is high, it induces rapid degradation of the enzyme as well as decreasing the amount of mRNA for HMG-CoA reductase which leads to decreased expression of the enzyme[12].

Cholesterol regulation of excess intracellular free cholesterol occurs by controlling the activity of ACAT (acyl-CoA:cholesterol acyltransferase), an enzyme which catalyzes the esterification of cholesterol and plays a role in intracellular cholesterol storage and hepatic lipoprotein assembly[13]. Inhibition of this enzyme lowers serum cholesterol and triglyceride levels and reduces hepatic production of apolipoprotein B[14]. Further regulation of plasma cholesterol levels occurs through LDL-R mediated uptake and HDL-C mediated reverse transport[15].

2.7: Pathway for lipid transport:

Chylomicrons and VLDL transports triglycerides from intestine to the skeletal muscle, adipose tissue or to the liver for synthesis of VLDL. VLDL is secreted from the liver to the circulation for transport to other tissues[16].

In the exogenous lipoprotein pathway, lipoproteins are synthesized by the intestine from dietary lipids whereas in the endogenous lipoprotein pathway lipoproteins are synthesized within the liver[17].

In the exogenous pathway the intestinal lipids are absorbed and assembled with apoB48, apoA-I, apoA-II and apoA-IV to make chylomicrons which are secreted to the lymphatic vessels and released directly into the circulation by the subclavian vein. Lipoprotein lipase removes triglycerol from chylomicrons and tissues absorb FFA and glycerol. After that triglycerides convert into chylomicrons remnant which make richer in cholesterol and cholesterol esters. These chylomicrons remnants are removed by the liver. Cholesterol may be used to form cell membrane or bile salts or may be excreted into the bile[16, 17].

The endogenous pathway composes transport of cholesterol and TGs from liver to muscle, adipose tissue in the form of VLDL. The lipoprotein particles become smaller but they increase in density to IDL cholesterol and ultimately LDL particles. Cells take up LDL by endocytosis through LDL receptors. Tissues get cholesterol in the form of HDL particles. After esterification of cholesterol, get changed to VLDL or LDL[16, 17].

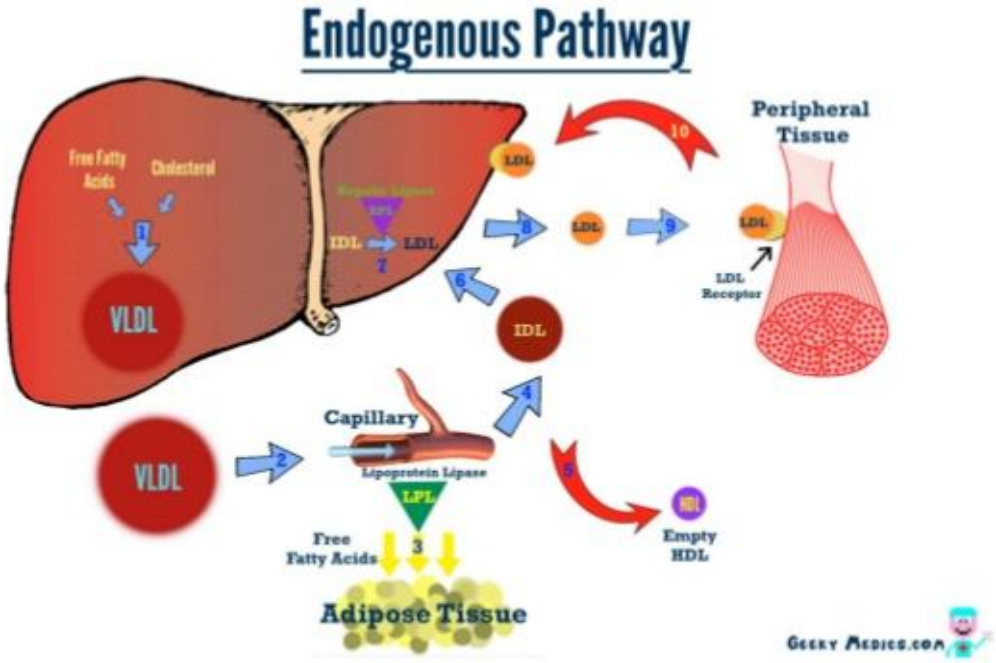


Figure: 2.3: Endogenous pathway,

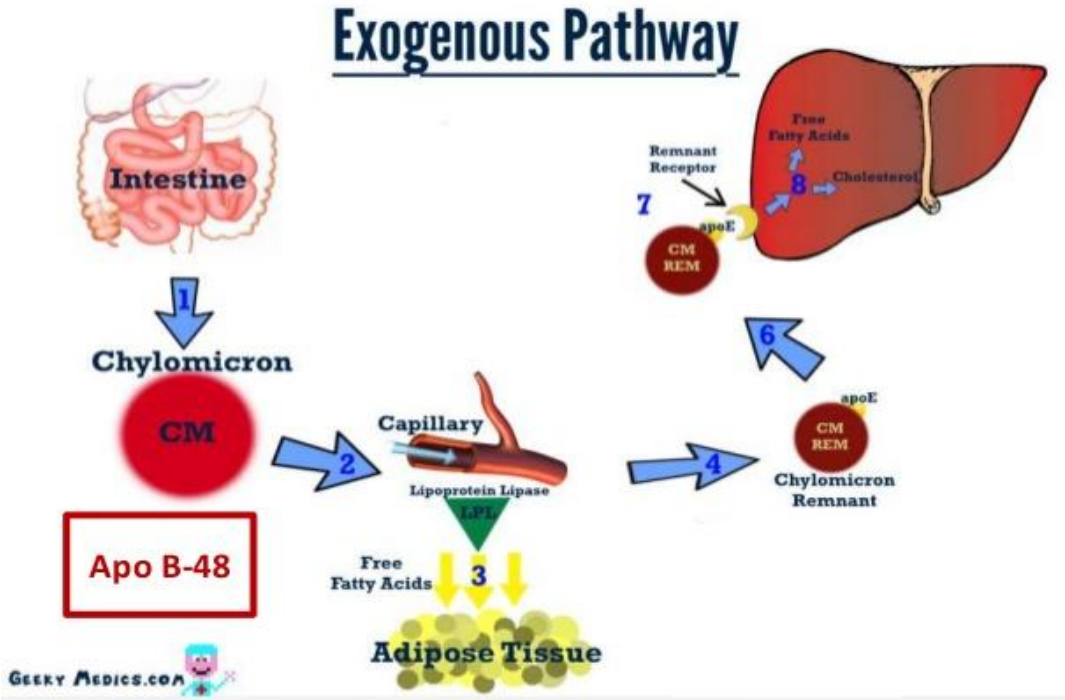


Figure: 2.4: Exogenous pathway

2.8: Dietary sources of fat:

The sources for the fat component may be either plant-derived or animal-derived[18]. Butter, ghee,various vegetable oils and fish oils, milk and milk products, egg, meat, pulses and cereals are the dietary sources of fats and lipids. All these foods contain different types of fatty acids like saturated, mono and poly unsaturated fatty acids in different ratios[1].

An ideal edible oil is one that contains all three types of fatty acids in equal ratios i.e. 1:1:1. Unfortunately there is no such single oil which contains all three types of fatty acids in equal ratios. Coconut oil, palm oil, ghee are rich in saturated fatty acids, ground nut oil and gingely oil are rich in mono saturated fatty acids, sunflower oil, cotton seed oil are on high content of poly unsaturated fatty acids[1].

2.9: Adverse effects of fats:

Mainly saturated fats produce positive energy balance and leads to increased fat deposition mainly in visceral organs. As the deposition of fat increases, it starts creating adipocyte stress and proliferation. All these pathologies may lead to various adverse conditions like obesity, dyslipidemia, atherogenesis, sympatho-vagal imbalance, cardiovascular diseases, altered glucose homeostasis, insulin resistance[19].

2.10: Micronutrient components of control and high fat diets:

Control diet is characterized by content of protein 18%, carbohydrate 62% , fat 20% and multivitamin and NaCl 2%, where as high fat diet contains high content of fats i.e. 30%, carbohydrate 52%, protein 18% and multivitamin and NaCl 2%[20].

2.11: Using rats as an experimental model for high fat diet: Because of ethical and pragmatic limitations for using human as a subject, animal models are usually preferred for

experimental research. Animal models are robust and reproducible subsequently at the end of dietary trial.

2.12: High fat diet as a trigger for various abnormalities (Pathologies):

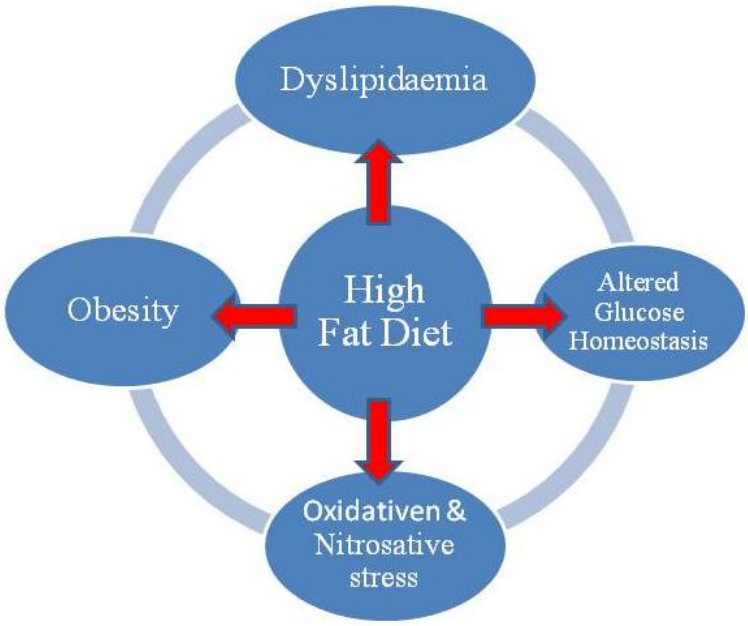


Figure: 2.5: High fat diet as trigger

2.13: Obesity:

Obesity is defined as the condition of abnormal or excessive fat accumulation in adipose tissue to such an extent that health may be adversely affected[21]. It is important risk factor for cluster of cardiometabolic abnormalities and have increased risk for development of obesity-related diseases, including cardiovascular diseases (CVDs) [22]

Obesity in rats: Obesity can be assessed in experimental rats without depending upon BMI classification. Animal obesity can be determined by few criteria like gain of body weight, % of body weight gain, organo somatic index and body fat content[5]. Many studies have reported that rats fed with hypercaloric diet have shown increased body weight and % body weight gain[23].

Obesity is very much associated to higher inflammatory state in white adipose tissue both in humans as well as rodents. Pathophysiology of obesity is initiated by activation of at least two inflammatory pathways such as JNK (Jun N-terminal kinase) and NF-kB (Nuclear factor kappa-light chain of activated B cells). Same modifications are visible in other insulin sensitive tissue such as liver, skeletal muscle, indicating connection between inflammation and development of insulin resistance[24, 25]. Obesity is closely associated with increased macrophase infiltration in the adipose tissue and will affect insulin sensitivity[26, 27].

Excess intake of high caloric diet and adipose tissue hypertrophy can cause excess secretion of chemokines which create chemotactic gradient and further attract monocytes. Later on these monocytes will become adipose tissue macrophages (ATM) which will release their own chemokines and attract more number of monocytes and initiate the inflammation process. Adipose tissue macrophages can accumulate lipids in adipose tissue in obese state and take foamy appearance. The increased number of macrophages and cytokine production increase the inflammatory status in the adipose tissue causing decreased metabolic and vascular functions[28].

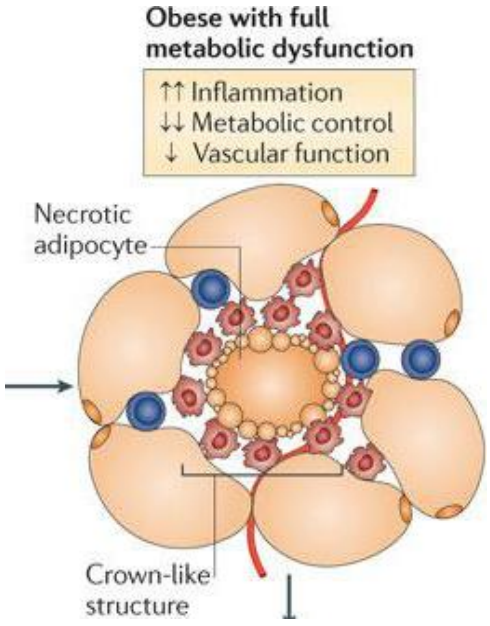


Figure: 2.6: Obese with full metabolic dysfunction

2.14: Vascular alterations in high fat diet:

High fat diet induced obesity stimulates metabolic and vascular alterations. The vascular abnormalities included an alteration in endothelial L-argenine/NO pathway[29]. The production and/or release of nitric oxide (NO), is an important endothelial factor involved in the regulation of vascular tone[30].

Endothelial NOS is commonly expressed in endothelial cells. Subsequently it is also found in cardiac myocytes, platelets, kidney tubular epithelial cells[31].

Endothelial NOS can synthesizes NO in a pulsatile manner with help of intracellular Ca^{2+}. These Ca2+ makes binding of calmodulin to the enzyme[32].

Regulation of endothelial NOS activity by intracellular Ca2+ and phosphorylation. An increase in intracellular Ca2+ leads to an enhanced binding of calmodulin (CaM) to the enzyme, which in turn displaces an auto-inhibitory loop and facilitates the flow of electrons from NADPH in the reductase domain to the haem in the oxygenase domain. Established functionally important phosphorylation sites in human endothelial NOS are Ser1177 and Thr495. In resting endothelial cells, Ser1177 is usually not phosphorylated. Phosphorylation is induced when the cells are exposed to oestrogens, vascular endothelial growth factor (VEGF), insulin, bradykinin or fluid shear stress. Endothelial NOS is potent regulator for numerous essential cardiovascular functions. Endothelial NOS-derived NO dilates all types of blood vessels by stimulating soluble guanylyl cyclase and increasing cyclic GMP in smooth muscle cells. Any kind of deletion of eNOS gene leads to elevated blood pressure. Nitric oxide released at vascular lumen is a potent inhibitor of platelet aggregation and adhesion to the vascular wall[33, 34]. It prevents the release of platelet-derived growth factors that stimulate smooth muscle proliferation and its production of matrix molecules. Endothelial NOS is also crucial for adaptive vascular remodeling to chronic

changes in flow. Subsequently NO inhibit DNA synthesis, mitogenesis and proliferation of vascular smooth muscle cells. Such kind of antipoliferative effects are likely to be mediated by cyclic GMP. The inhibition of platelet aggregation and adhesion reserves smooth muscle from exposure to platelet-derived growth factor. Hence, NO also prevents a later step in atherogenesis, fibrous plaque formation[35, 36, 37].

2.15: *Role of eNOS in pathophysiology*

Patients with cardiovascular risk factors like hypertension, hypercholesterolaemia, diabetes mellitus, cigarette smoking and patients with vascular disease represent with endothelial dysfunction, i.e. the inability of the endothelium to generate sufficient amounts of bioactive NO (and to produce NO-mediated vasodilatation). These cardiovascular risk factors and vascular diseases are linked with an increased production of ROS. Oxidative stress converts eNOS from NO producing enzyme to an enzyme which generates O_2[38].

2.16: High fat diet and dyslipidemia:

Dyslipidemia is the condition where body is unable to maintain its pool of lipids within optimal physiological ranges[15]. It is a state characterized by higher levels of triglycerides, lower levels of HDL and abnormal free fatty acids (FFA) due to increased release by the enlarged adipose tissue and reduction of FFA clearance[5].

Pathophysiology of dyslipidemia: dyslipidemia occurs mainly due to disturbed regulatory system which changes functioning of various proteins involved in lipometabolic pathways. Ultimately it leads to imbalance between lipid biosynthesis and clearance[15]. There occurs defect in lipid metabolism due to lack of lipoprotein lipase enzyme and or absence of the surface apoprotein CII. It is also associated with increased free fatty acids (FFA) levels, due to their increased release by the proliferated adipose tissue and reduction of FFA clearance.

Subsequently increased levels of FFA inhibit insulin's antilipolytic action, which will further increase FFA release into the circulation[5].

2.17: High fat diet and glucose homeostasis:

Fatty acid synthesis and oxidation, cholesterol and bile acid synthesis and lipoprotein accumulation are metabolic pathways regulated by the liver to maintain homeostatic control of both glucose and lipid levels in plasma[39].

Hallmark events occurring in relation with fat diet to glucose homeostasis are plasma FFA, ectopic lipid accumulation in metabolic organs, adipose tissue, peripheral insulin resistance, hyperglycemia and pancreatic dysfunction[5].

In experimental animals dietary high fats causes impaired glucose tolerance. This impairment is mainly related to decrease basal and insulin stimulated glucose metabolism. Mainly saturated fats compared to mono or polyunsaturated fats are more damaging in relation to insulin insensitivity. Epidemiological data reports that more consumption of high fat diet are more prone for disturbed glucose metabolism, type 2 diabetes or glucose intolerance[40].

Role of liver in glucose homeostasis:

Liver is an important organ which maintains balance between glucose release and uptake. In fasting condition liver generates glucose by gluconeogenesis and glycogenolysis and in fed condition liver uses glucose from circulation and stores as glycogen. Hence liver plays an important role in maintaining glucose homeostasis[41].

Role of Insulin:

Insulin is the prime hormone to control over glucose metabolism although its actions are altered in various ways by counter regulatory hormones such as adrenaline, glucagon, growth hormone and cortisol. Insulin maintains harmonized interaction between various tissues to

achieve equilibrium between glucose output and uptake[41]. Insulin is synthesized in beta cells of pancreas and stored in the cytosole in secretary granules. Insulin is secreted by pancreatic beta cells in response to elevated levels of nutrients like increased circulating levels of fatty acids and glucose after meal. Glucose can't enter into cell directly, for which it needs one of two glucose transporter mechanisms like Na+-dependent-glucose transporters (SGLTs) and Na+-independent glucose transporters (GLUTs)[41]. Mainly glucose enters into the beta cells of pancreas through GLUT 2 transporters by facilitated diffusion. Metabolism of glucose occurs in the beta cells of pancreas and production of ATP occurs over there. It leads to increase in ATP/ADP ratio in cytoplasm and causes closure of ATP sensitive potassium channels. Subsequently it leads to depolarization of plasma membrane and opens voltage gated ca^{2+}, and influx of extracellular calcium into the cell. This increased levels of ca^{2+} causes release of insulin from the beta cells of pancreas by exocytosis[42].

Insulin resistance:

In this condition cells become resistant to the effects of insulin, leading to insufficient levels of physiological insulin to regulate glucose homeostasis by peripheral target tissues. There will be altered glucose homeostasis in target tissues such as liver, skeletal muscle and adipose tissue. In liver, it causes decreased glycogen synthesis and storage and failure to reduce glucose output into the blood. Insulin resistance in extra hepatic tissues leads to decrease in glucose uptake and storage of glucose as glycogen and triglycerides. Initially β-cells compensate for insulin resistance by generating excess amounts of insulin, so that normal glucose tolerance is maintained. Despite increased insulin secretion from the β-cells becomes less effective in lowering plasma glucose levels. Eventually, the secretion of insulin reaches a point where it is impossible to counteract the insulin resistance[41].

Rats fed with high fat diet have significantly gained weight and have higher blood glucose and triglyceride levels. Hyperglycemia is attributed to impaired disposal of glucose by peripheral tissues due to lesser sensitivity to endogenous insulin[40]. It was also suggested that high fat feeding induces reduction in glucose uptake and oxidation and/or reduction in conversion to fatty acids which is considered as secondary to decreased insulin sensitivity[40].

It was also mentioned that high fat fed rats resulted in decrease in GLUT 4 protein concentration as per the adipocyte than glucose transport rate. It clearly indicates that alteration in GLUT 4 expression is directly related to hypoinsulinaemia. Impaired insulin binding and /or glucose transporters have been associated to changes in the fatty acid composition of the membrane induced by dietary fat modification. Fatty acid composition of the membranes of peripheral tissues affects insulin sensitivity. The possible mechanisms include the fluidity of cell membrane, the number and affinity of insulin receptors alters the activities of enzymes associated with glucose metabolism[40].

2.18: High fat diet and oxidative stress:

Oxidative stress is defined as a continuous imbalance between generation of reactive oxygen species (ROS) and antioxidant defenses[43]. These ROS cause tissue damage as these are highly reactive atoms. They can alter the molecular structures like lipids, proteins and DNA. ROS contains unstable oxygen radical (O_2-) like superoxide radical, singlet oxygen and hydroxyl radical also non radical molecules like hydrogen peroxide[44]. Oxidative stress is related to pathological conditions like obesity, diabetes and various cardiovascular diseases[45]. ROS are generated intracellular pathway via variety of processes, like byproducts of normal aerobic metabolism, or as second messengers in signal transduction pathways. They can also be produced from exogenous sources and being taken up directly by cells from the extracellular environment. Finally they can be derived as a result of the cell exposure to environmental insult[5].

Reactive nitrogen species (RNS) are a family of antimicrobial molecules derived from nitric oxide ($NO^{\bullet}$) and superoxide ($O2^{\bullet-}$) generated through the enzymatic activity of inducible nitric oxide synthase 2 (NOS2/iNOS) and NADPH oxidase. RNS acts along with ROS to damage cells, leading to nitrosative stress. A number of defense systems have developed to fight against the accumulation of ROS. These include non-enzymatic molecules such as glutathione, vitamins C and E and flavonoids also enzymatic ROS scavengers like SOD, CAT and GSH. Unfortunately, these defense mechanisms are not always sufficient enough to counteract the generation of ROS resulting in a state of oxidative stress[5].

There is increasing evidence that high fat diet induced oxidative stress is related to increased risk for cardiovascular diseases, vascular damages and altered glucose homeostasis[46]. Mainly these conditions are characterized by increased production of ROS, endothelial dysfunction and decreased NO bioavailability[47].

In experimental animals infusion of high fat diet is associated with elevated levels of FFA and fat mass expansion[48]. Subsequently it leads to oxidative stress and insulin resistance. Elevated levels of FFAs cause oxidative stress through increased beta-oxidation of cytosolic long chain acyl-coA esters. FFAs can also stimulate ROS production by activation of NAD(P)H oxidase in aortic smooth muscle cells and endothelial cells[49]. Oxidative stress enhances susceptibility of increased lipid pools to lipid oxidation by eliciting lipid Peroxidation[50].

Mitochondria are the most important cellular source of ROS, which oxidize the saturated lipids of fat deposits to cause lipid Peroxidation. These intermediates react with oxygen to form large number of superoxide anion and other ROS. Lipid Peroxidation ad ROS ingest antioxidant enzyme and vitamins. This HFD - induced ROS directed pro-inflammatory state may activate

one of the major transcription factor linked with inflammation, NF-κB. Moreover, either this HFD triggers ROS or NF-κB induces the expression of NF-κB-dependent pro-inflammatory agents like inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF-α) interferon γ (IFN-γ) .Various enzymes inside the cells are involved in oxidative stress mechanism but NADPH oxidase is an important source of ROS generation. NADPH oxidase is a membrane bound enzyme which transfer electrons from NADPH to oxygen. This generated oxygen O_2- will convert into H2O2 mainly by superoxide dismutase (SOD)[51].

Nitrosative stress:

Nitric oxide is a small molecule which contains one unpaired electron and it is produced in biological tissues by nitric oxide synthases (NOSs). NO is an reactive radical which acts as an important oxidative biological signaling molecule in a large variety of physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation[5].

NOS is an important enzyme causing oxidative stress at endothelial cells[49]. Main adverse effect of ROS on endothelial cells is that it shows decreased bioavailability of NO, resulting from eNOS uncoupling. Perivascular adipose tissue increases vascular contractile responses by inhibiting endothelium dependent vasodilatation via restricting endothelial NOS (eNOS)[52]. Recently it has been reported that high fat diet induced rats have shown endothelial dysfunction through increased NADPH oxidase derived oxidative stress and production of proinflammatory cytokines. Altered angiogenic process occurred due to changed $NO^{\cdot-}$ involve in inadequate vasculature observed upon fat expansion giving rise to hypoxia[53].

Hence, ROS-induced endothelial dysfunction will not only impair blood flow regulation but also restrict capillary network formation. Such alterations will result ultimately in attenuation

of microcirculatory network in metabolic active tissues and eventually decrease in glucose utilization[54].

Intake of antioxidants such as vitamins C, E and beta carotene through diets or fruits may reduce risk of oxidative stress. Also various Polyphenols have pointed out its beneficial effects as an antioxidant and free radical scavengers. Polyphenols mainly contains various pytochemicals derived from the extraction of medicinal plants, fruits, seeds and vegetables. Its antioxidant activity mainly depends on inhibition of ROS generating enzymes along with up regulation of multiple antioxidant enzymes[55].

2.19: High fat diet and Autonomic functions:

Diet is one lifestyle factor which can either help or damage cardiac function, depending on the types of foods consumed by the individual. High consumption of fish, vegetables, and fruit generally leads to beneficial changes in HRV. In addition, there is a significant relation between high cholesterol and low HRV. High level of LDL cholesterol in the body is also a main risk factor for heart disease. Therefore, HRV is a best tool to provide how nutrition and diet play a critical role in cardiac health[56].

Heart rate variability is defined as beat to beat variation in duration of R-R interval[57]. HRV is the physiological phenomenon which accounts the overall health of the heart and can assess the cardiac health[58].

HRV is considered as most reliable measure of cardiac electrophysiology and autonomic function. It is due to the fact that cardiac rhythm is under the influence of minute to minute and circadian effect of the autonomic nervous system. A balance between the sympathetic and parasympathetic components of ANS gives normal patterns of HRV[59]. High variability in heart rate is a mark of good adaptability, and lower variability is a sign of abnormal adaptability of the

ANS, indicating vulnerable pathology. Hence HRV analysis is non invasive method of giving information about cardiac autonomic modulations[60].

Measurement of HRV:

The initial step for HRV recording is getting high quality ECG under stationary conditions. These ECG signals are digitally modified for computer processing in terms to get good quality time resolution, a sampling rate at least up to 1000Hz especially for rat ECG signals is referred. According to definition of HRV it is time between consecutive R wave peaks. Mainly there are three main categories to measure HRV i.e. time domain, frequency domain and non-linear dynamics methods[60].

Time Domain:

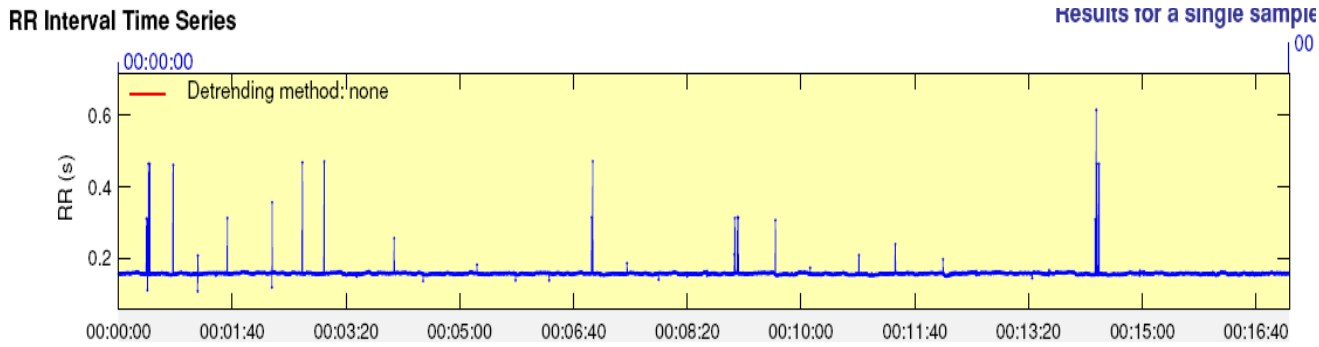


Figure: 2.7: HRV; courtesy laboratory of vascular physiology and medicine

It is the simplest method to record HRV in a specific time period in a continuous ECG signal. Standard deviation and Root mean square of successive differences between adjacent R-R intervals are most frequently used time domain indices[60].

Standard deviation of R-R interval is the square root variance. It quantifies the balance between sympathetic and parasympathetic control of heart rate i.e it measures overall heart rate variability. Root mean square of successive differences between adjacent R-R intervals is the

difference between successive R-R intervals is summed, the result averaged and then the square root obtained. It gives very short-term HRV measured over a much longer period of time[60].

Frequency domain:

Spectral analysis decomposes any time-dependent changing signal into its sinusoidal components and measures the amount of cyclical variation present at different frequencies[61]. Graph is plotted such as, the amount of variation present in a recording on the vertical axis against the frequency at which occurs on the horizontal axis. The graph is labeled as power spectrum. There are two methods to measure algorithms i.e non parametric and parametric methods. Most frequently used non parametric algorithm is fast Fourier transform (FFT). Most commonly used parametric algorithm is autoregressive modeling (AR). The AR algorithm calculates automatically low- and high- frequency power components with an easy identification of the central frequency of each component. It is very beneficial to get a reliable and correct spectral estimation even with short segments of data. In particular spectral curve, three frequency bands i.e. Very low frequency (VLF), low frequency (LF) and high frequency (HF)[60].

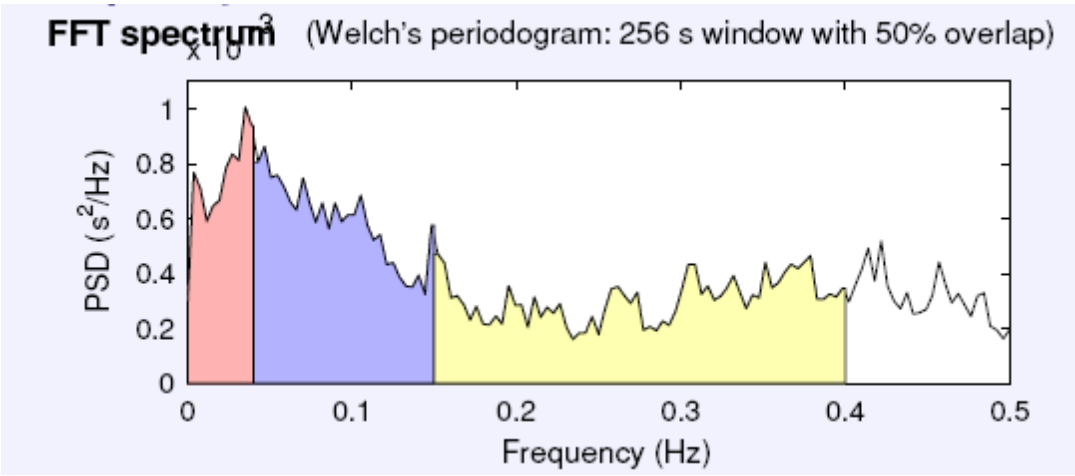


Figure: 2.8: HRV; courtesy laboratory of vascular physiology and medicine

LF is the indicator of sympathetic activity and HF is the marker of parasympathetic activity. LF/HF ratio quantifies the fractional distribution of power, which is considered as an indirect measure of sympatho-vagal balance[60].

Applications of HRV:

Application of HRV is very beneficial in determining role of ANS in modulation of cardiovascular response during many physiological functions. Such as when there is change in body posture from supine to standing, there is shift of sympatho-vagal balance from parasympathetic dominance to sympathetic dominance. It is reflected by corresponding changes in HEV variability indexes. HRV analysis has been also reported role of the ANS in regulating cardiovascular circadian rhythms, showing sympathetic predominance in the morning and parasympathetic dominance in the night[62].

Autonomic dysfunction plays an important role in the pathophysiology of ischaemic heart disease and hypertension. Autonomic dysfunction related with decreased HRV is a strong risk factor for overall mortality, ventricular tachycardia, and sudden death following myocardial infarction[63].

Modern medications against hyperlipidemea with contraindications:

Nowadays there are various classes of drugs which are used to cure patients with adverse effects of high lipid levels. Among them five classes of drugs are more popularly used those are statins, nicotinic acid derivatives, bile acid binding resins and cholesterol absorption inhibitors[64,65].

Among them statins is the drug of choice most commonly used. The potent members of statins class of drugs include atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and Simvastatin[66, 67]. These are also known as 3-hydroxy 3-methyl glutaryl CoA (HMG-CoA)

reductase inhibitors because they have been reported to inhibit the rate at which cholesterol is formed in the body[68].

But few associated complaints have been reported such as stomach upset, nausea, vomiting, headache and dizziness. Next class of drug of choice is Niacin which affects cholesterol formation and decreased production of triglycerides in the body. The common side effects of niacin drugs are flushing, hot flashes, itching and headache[69]. Next potent hypolipidemic agent is fibric acid derivatives which decreases formation of cholesterol and triglycerides in the body. Reported side effects of this class of drugs are heart burn and stomach pain[70].

Need for Novel natural agent in modulating hyperlipidemia and altered glucose homeostasis due to high fat diet

As above mentioned therapy is considered as long term treatment, there may be risk of chronic effects like muscle toxicity, carcinogenic and mutagenic. Hence it is need of the hour to explore natural source of medicines those are less toxic, less expensive which can provide better safety and efficacy on a long term usage. Ayurvedic system of medicine consists of various herbal drugs. Many herbal drugs have been reported to be useful in the treatment of cardiovascular diseases including hyperlipidemea. Among these herbal drugs *Emblica Officinalis* (Amla) occupy an important place in the context of such medicinal values. Recently there has been renewed interest in these plants because of its multimode cardioprotective activity[71].

Figure: 2.9.A: Amla fruits

Figure 2.9.B: Amla fruits and tree

2.20: Plant profile of *Emblica Officinalis (Amla)* with its biological activities[72]:

Plant name: *Emblica Officinalis* (Amla)

Kingdom- Plantae

Division- Angiospermae

Class- Dicotyledonae

Order- Geraniales

Family- Euphorbiaceae

Gene- Emblica

Species- *Officinalis* geartn**.**

Regional names:

English: Emblic myrobalan, Indian goose berry

Sanskrit: Aamalaki, **Hindi:** Amla, **Kannada:** Nelli Kayi, **Marathi:** Avla, **Gujarati:** Ambla, **Malayalam:** Nelli Kayi, **Tamil**: Nelli, **Telugu:** Usirikaya and **Kashmir:** Aonla.

2.21: Description of Amla: Amla is the medium sized deciduous tree. It grows to the height of 8-10 meter with edible fruit belongs same name. it has a crooked trunk with spreading branches. Its flowers are yellow and greenish in colour. The genus name phyllanthus is derived from Greek word which means leaf flower an allusion to the apparent bearing of flowers on the leaves. The

fruit is spherical, pale yellow in colour with six vertical furrows. The taste of Amla is sour, bitter and astringent, and is somewhat fibrous. The average weight of fruit is 60-70gms.leaves are linear, feathery, oblong in shape and smell like lemon[73].

Geographical distribution:

It is planted through the deciduous of tropical India and on the hill slopes up to 2000 meter. It is commercially cultivated in the state of Uttar Pradesh in India. It is also grown in Tamil Nadu, Rajasthan and Madhya Pradesh and Maharashtra also. Grows in tropical and subtropical parts of Ceylon, Malay Peninsula,Pakistan, Bangaladesh, Shrilanka and China. In Ceylon, it is very common in exposed places on Patana land in the moist regions up to 4000 feet altitude[74].

Harvesting and post harvesting:

Amla tree strats bearing fruits in 7-8 years after planting. Best harvesting time of Amla fruits is from November- February, at this time fruits have maximum ascorbic acid content. In south India fruits are found through year. The mature fruits are hard and needs vigorous shaking for fall of fruits[72].

Yield:

Almost 50-70kg of fruits will yield to a mature tree of 10 years. A well maintained tree will be yielding upto an age of 70 years. The yield increases year by year up to 50 years[72].

2.22: Chemical constituent of Amla:

This herb has many substances, including apigenin, gallic acid, ellagic acid, chebulinic acid, quercetin), chebulagic acid, corilagin, isostrictiniin, methyl gallate, and luteolin. Tannins in amla include Phyllaemblicin B, emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin. Amla is highly nutritious and is an important dietary source of Vitamin C,

minerals and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples. Glutamic acid, proline, aspartic acid, alanine, and lysine are 29.6%, 14.6%, 8.1%, 5.4% and 5.3% respectively of the total amino acids. The pulpy portion of fruit, dried and freed from the nuts contains: gallic acid 1.32%, tannin, gum 13.75%; albumin 13.08%; crude cellulose 17.08%; mineral matter 4.12% and moisture 3.83%. Amla fruit ash contains chromium, 2.5 ppm; zinc 4 ppm; and copper, 3 ppm[75].

2.23: Ethno medical uses of Amla:

Emblica enjoys a hallowed position in Ayurveda, an indigenous system of medicine in India[76]. It is proved to be effective against diabetes, cough, asthma, bronchitis, dyspepsia, colitis, hyper acidity, peptic ulcer, skin diseases, inflammations, anemia, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhage, leucorrhoea, cardiac disorders, intermittent fevers and greying of hair and is given to cancer. It is also a very good blood purifier which in turn improves the health of liver by keeping the toxins and infections away. It is also used as antioxidant,cardio protective, strengthen heart and hypolipidemic[76].

2.24: Role of Amla in dyslipidemia:

In hyperlipidemic rat model, Amla has shown significant changes in lipid profile may be due to several mechanisms such as an interference with cholesterol absorption[77]. Flavonoids present in Amla significantly lowered lipid levels in serum and tissues by its inhibitory action on HMG CO-A reductase pathway[78].

Also standardized extract of Amla fruit increases cardiac glycogen levels and decreases LDL levels. The mechanism may include increase in LCAT (Lecithin Cholesterol acyltrans): the enzyme responsible for ester transfer to HDL. Similarly there may be increase in lipoprotein

lipase activity. This increased enzymatic activity increases the ability of muscle fibers to oxidize fatty acids coming from VLDL, TG. This process of cholesterol removal from circulation is termed as reverse cholesterol transport[79].

2.25: Cardio protective activity of Amla:

It was reported that an increase in the cardiac glycogen suggesting a cardio protective action of amla. It was mentioned that Polyphenols present in amla fruit juice (541.3 mg gallic acid equivalent / 1 gm extraction) might be responsible for lipid lowering effects of fruit juice which may explore cardio protective effects[80]. Hypercholesterolemia is associated with decreased 24 hour heart rate variability. Decreased heart rate variability is a valuable predictor of CHD[81]. Chronic *Emblica Officinalis* (Amla) administration produces myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative stress.

2.26: Amla as an antioxidant:

It has been well documented from various studies that Amla is a powerful antioxidant. *E.officinalis* reduced the oxidative stress by its free radical scavenging property. This antioxidant potential has been attributed to flavonoid contents of *E.offficinalis* [82]. Supplementation of EEO to hyperlipidemic rats showed significant increase in the activity of superoxide-dismutase, catalase, glutathione and ascorbic acid. Vitamin C present in the extract of Amla maintains the first natural antioxidant defense activity and acts as a powerful inhibitor of lipid peroxidation[83]. Possibly, emblicanin A of EEO aggressively seeks and attacks free radicals. After it neutralizes a free radical, Emblicanin A is transformed into Emblicanin B, another antioxidant. Emblicanin B in turn attacks free radicals and is transformed into emblicanin oligomers thus prevent further oxidative damages[82]. It has also been noted that phenolic content in ethanolic extract of Amla has shown antioxidant potential by inhibiting auto-oxidation via free radical scavenging, singlet oxygen quenching and hydrogen donating mechanisms[83]. Possibly ellagic acid and ascorbic acid

in extracts of Amla found to accelerate antioxidant property by increase in nitric oxide and decrease in hydroxyl radicals through its free radical scavenging property and prevention of LDL oxidation in our study[84, 85]. Unaltered NOS3 protein with decreased NO in high fat fed rats indicate a possible alteration of vascular pathophysiology probably through oxygen sensing cell signaling pathway[86].

2.27: Role of Amla in altered glucose homeostasis:

High fat diet is associated with insulin resistance and reduced insulin secretion by beta cells in the pancreas which may lead to altered glucose homeostasis[87].The possible link between hyperlipidemea and beta cell dysfunction of pancreas may be due to elevated plasma Free Fatty Acids (FFA). Lipotoxicity caused by elevation of FFA induces diabetogenic effect[3]. *Emblica officinalis* supplementation is effective in reducing blood glucose levels by regenerating and rejuvenating beta cells of pancreas and increasing insulin production and secretion[80]. *Emblica officinalis* is rich in polyphenolic contents (541.3 mg gallic acid equivalent/1 gm extract) might have glucose lowering effects[88]. Tannoids present in *Emblica officinalis* extract act as a strong inhibitors of aldose reductase[84].

2.28: Hepato protective effect of Amla:

The fruit of *Emblica Officinalis* is a considered as major constituent of liver tonic used against acute viral hepatitis and various liver disorders[89].

V Damodar Reddy reported that after administration *of Emblica Officinalis* fruit extract to rats treated to alcohol improved the histomorphology of the liver near to normal. The *Emblica Officinalis* fruit extract supplementation to rats indicates the hepatoprotective role also preserves the structural integrity of the liver from the adverse effects of alcohol[90].

REFERENCES:

1) Rafi M D. Text book of Biochemistry. 3rd Ed. published by universities press 2014. Chapter 16 metabolism of lipids P:221.

2) FREDRIK ROSQVISTDietary Fatty Acids, Body Composition and Ectopic Fat Results from Overfeeding Studies in Humans

3) Picchi MG,Mattos AM, Barbosa MR, Durate CP, Gandini MD, Portari GV et al. A high fat diet as a model of fatty liver disease in rats. Acta Cir Bras2011; 26(2):25-30.

4) Wong SK, Chin KY,Suhaimi FH, Fairus A, Ima Nirwana S. Animal model of metabolic syndrome : a review. Nutrtion & metabolism.2016Dec1;13(1):65.

5) Mourmoura E. Les effets de l'augmentation de la masse adipeuse sur la fonction cardiovasculaire ex vivo en fonction du stress oxydant et de la fonction mitochondriale: rôle du vieillissement du régime alimentaire (Doctoral dissertation, Université de Grenoble).

6) Kyu Yol Edward Park.The mechanisms underlying free fatty acid-induced hepatic insulin resistance. 2008 University of Toronto.

7) Demaison L, Moreau D, Vergely-Vandriesse C, Gergoire S, Degois M, Rochette L. Effects of dietry polyunsaturated fatty acids and hepatic steatosis on the functioning of isolated working rat heart under normoxic conditions and during post ischaemic reperfusion. Molecular and cellular biochemistry 2001; 224(1-2):103-116.

8) Wang P, Lloyd SG, Zeng H, Bonen A, Chatham JC. Impact of altered substrate utilization on cardiac function in isolated hearts from Zucker diabeteic fatty rats. Am J Physiol Heart Circ Physiol 2005; 288(5):2102-10.

9) Meyes PA, Botham KM. Lipids of Physologic significance. In: Murray RK, Garner DK, Mayes PA, Rodell VW editors. Harper's Illustrated Biochemistry. 26th ed. New York: Lange Medical Books:2003. P111-22.

10) Jain KS, Kathiravan MK, Somani RS, Shishoo CJ. The biology & Chemistry of Hyperlipidemia. Bio-organic and medicinal chemistry 2007;15:4674-99.

11) Bjermo H. Dietary Fatty acids and inflammation: observational and interventional studies. 2011; Doctoral dissertation at Uppsala University,

12) Osborne TF. Transcriptional control mechanisms in the regulation of cholesterol balance. Crit Rev.Eukaryot.Gene Expr.1995; 5:317-35.

13) Chang TY. Acyl-coenzyme A: Cholestrol acyltransferase. Annu. Rev.Biochem 1997;66:613-38.

14) Burnett JR. Inhibition of cholesterol esterification by DuP 128 decreases hepatic apoliprotein B secretion in vivo: effect of dietary fat and cholesterol. Biochim. Biophys.Acta 1998;1393:63-79.

15) Gloria Woo. Secoisolariciresinol diglucoside effects in diet induced hyperlipidemic rats. University of Saskatchewan Saskatoon.

16) Pankaja Naik. Lipid metabolism. In Text Book of Biochemistry.2nd ed.Jaypee Medical Publisher; 2007:265-18

17) Satyanarayan U. Lipid Metabolism. 2nd ed.Vijayawada. Uppala author-publishers interlinks; 2002.

18) Wong Sk, Chin KY, Suhaimi F, Fairus A, Nirwana SI. Animal model of metabolic syndrome: a review. Nutr Metab 2016:13-65.

19) Handayani D. Prevention and treatment of high fat diet-induced obesity by dietary intake of Shiitake and oat. 2012; University of Wollongong.

20) Das K K, Dasgupta S. Effect of Nickel Sulfate on testicular steroidogenesis in rats during protein restriction. Environmental Health Perspectives. 2012; 110 (9):923-26.

21) Swinburn B, Egger G, Raza F. Dissecting obesogenic environments: the development and application of a framework for identifying and prioritizing environmental interventions for obesity. Prev. Med. 1999:563-70.

22) Chiu HC, Kovacs A, Ford DA et al. A novel mouse model of lipotoxic cardiomyopathy. J Clin Invest 2001; 107: 813-22.

23) Lopaschuk GD, Folmes CD, Stanley WC. Cardiac energy metabolism in obesity. Cir Res 2007;101:335-47.

24) Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. Nature 2002;420(6913):333-6.

25) Yaun M, Konstantopoulous N, Lee J, et al. reversal of obesity and diet induced insulin resistance with salicyclates or targated disruption of ikkbeta. Science 2001; 293(53):1673-7.

26) Helilbronn LK, Champbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. Curr Pharm Des 2008; 14(12):1225-30.

27) Oliver E, Mc Gillicuddy F, Phillips C, Toomey S, Roche HM.the role of inflammation and macrophase accumulation in the development of obesity induced type 2 diabetes mellitus and the possible therapeutics effects of long chain n-3 PUFA. Proceeding of the Nutrition Society 2010; 69(02):232-43

28) Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11(2):85-97.

29) Nascimento TB, Baptista RD, Pereira PC, et al. Vascular alterations in high fat diet obese rats: role of endothelial L-arginine/NO pathway. Arquivos brasileiros de Cardiologis 2011; 97(1):40-5.

30) Widlansky ME, Sesso HD, Rexrode KM, Manson JE, Gaziano MJ. Body mass index and total and ardiovascular mortality in men with a history of cardiovascular disease. Arch Intern Med 2004; 164(21):2326-32.

31) Forstermann U, Closs EI, Pollock JS, Nakane M et al. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning and functions. Hypertension 1994; 23:1121-31.

32) Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. European heart journal 2011; 33(7):829-37.

33) Alheid U, Frolich JC, Forstermann U. Endothelium derived relaxing factor from cultured human endothelial cells inhibits aggregation of human platelets. Thromb Res 1987; 47:561-71.

34) Radomski MW, Palmer RM, Moncada S. The anti aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol. 1987;92:639-646.

35) Garg UC, hassid A. Nitric oxide generating vasodilators and 8-bromo cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 1989; 83:1774-77.

36) Nakaki T, Nakayama M, Kato R. inhibition by nitric oxide and nitric oxideproducing vasodilators of DNA synthesis in vascular smooth muscle cells. Eur. J Phrmacol 1990;189:347-53.

37) Southgate K, Newby AC. Serum induced proliferation of rabbit aortic smooth muscle cells from the contractile state is inhibited by 8-Br-cAMP but not 8-Br-cGMP. Atherosclerosis 1990; 82:113-23.

38) Mueller CF, Laude K, McNally JS, Harrison DG. Redox mechanism in blood vessels. Arterioscler Thromb Vasc Biol 2005;25:274-78.

39) Evan Fjaere. Effects of macronutrient composition and cyclooxygenase inhibition on diet induced obesity, low grade inflammation and glucose homeostasis. 2012 University of Copenhagen, Copenhagen.

40) Linchenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis 2000 Jun;150 (2): 227-43.

41) Yassin K. Studies on factors modulating glucose homeostasis in healthy and diabetic rats. Dept of molecular medicine and surgery;2011 Sep 29.

42) Gondwe MM. Hypoglycaemic effects of some medicinal plant extracts in streptozotocin induced diabetic rats. 2007; University of Kwazulu, Natal.

43) Evan JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidatstress activated signaling pathways mediators of insulin resistance and beta cell dysfunction? Diabetes 2003; 52:1-8.

44) Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408(6809):239-47.

45) Pihl E et al. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athlets. Int. J Obes (Lond) 2006; 30(1):141-6.

46) Hopps E, Noto D, Caimi G, Averna MR. A novel component of the metabolic syndrome: the oxidative stress. Nutrition, Metabolism and cardiovascular diseases 2010; 20(10):72-77.

47) Galili O, Versari D, Sattler KJ. Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress. American Journal of Physiology-Heart and Circulatory Physiology 2007; 292(2):904-11.

48) Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM. Influence of body fat distribution on free fatty acid metabolism in obesity. Journal of Clinical investigation 1989; 83(4):1168-73.

49) Inoguchi T, LiP, Umeda F, et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes; 49(11):1939-45.

50) Russell AP,Gastaldi G, Bobbioni Harsch E, et al. Lipid Peroxidation in skeletal muscle of obese as compared to endurancetrained humans: a case of good vs bad lipids? FEBS Letters 2003; 551(1-3):104-06.

51) Le Lay S, Simard G MC, Andriantsitohaina R. oxidative stress and metabolic pathologies:from an adipocentric point of view. Oxidative medicine and cellular longevity 2014 Jul 20;2014.

52) Payne GA, Bohlen HG, Dincer UD, Borbouse L, Tune JD. Periadventitial adipose tissue impairs coronary endothelial function via PKC ß-dependent phosphorylation of nitric oxide synthase. The American journal of Physiology- heart and Circulatory Physiology 2009; 297(1):46065.

53) Hosogai N, Fukuhara A, Oshima K et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007; 56(4):901-11.

54) V'asquez VJ, Kalyanaraman B, Mart'asek P et al. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proceedings of the National Academy of Sciences of the United States of America 1998; 95(16):9220-25.

55) Andriantsitohaina R, Duluc LJ, Garc'la-Rodrguez et al. Systems biology of antioxidants. Clinical Science 2012 ;123(3):173-92.

56) Murray OA. The correlation between heart rate variability and diet. Proceedings of the National Conference on Undergraduate Research (NCUR) 2016:1185-91.

57) Rono DK. Effect of variable high fat diets on heart rate variability and selected modifiable cardiovascular risk factors.2013; University of Nairobi.

58) Stein PK, Kleiger RE. Insights from the study of heart rate variability. Annual Review of Medicine 1999;50: 249-61.

59) Zhang Y, Mazgalev T. Arrhythmias and vagus nerve stimulation. Heart Fail.Rev;16(2):147-61.

60) Trombini M. Heart rate variability in animal models of psychological cardiovascular comorbity. 2012; Doctoral dissertation Universita degi Studi di Parma. Dipartimento di Biologia evolutiva evolutiva e funzionale.

61)Malliani A Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation: Research Advanced Series 1991; 84:482-92.

62)Ewing DJ, Neilson JMM, Shapiro CM, Stewart JA, Reid W. 24-h heart rate variability:effects of posture, sleep and time of day in normal subjects and comparision with bedside function tests in diabetic patients. Br Heart J 1991;6: 239-44.

63) Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. reduced heart rate variability and new onset hypertension: insights into pathogenesis of hypertension. The Faminghan Heart study. Hypertension 1998; 32:293-97.

64) Rosuvastatin-a new lipid lowering drug. Med Lett Drugs Ther 2003;45:81-3.

65)Braamskamp MJ, Wilburg FA, Wiegman A. Drug therapy of hypercholesterolemia in children and adolscents. Drugs 2012; 72:759-72.

66) Davidson MH. Safety profiles for the HMG-CoA reductase inhibitors. Drugs 2001;61:197-206.

67) Koh KK, Quon MJ, Rosenson RS, Chung WJ, Han SH. Vascular and metabolic effects of treatment of combined hyperlipidemia; focus on statins and fibrates. Int J Cardiol 2008;124;149-59.

68) Hou R, Goldberg AC. Lowering low density lipoprotein cholesterol:statins, ezetimibe, bile acid sequestrants and combinations: comparative efficacy and safety. Endocrinol Metab Clin North Am 2009; 38:79-97.

69) Guyton JR, Brown BG, Fazio S, Polis A, Tomassini JE, Tershakoyec AM. Lipid altering efficacy and safety of ezetimibe/Simvastatin coadministered with extended release niacin in patients with type II a or type IIb hyperlipidemia. J Am Coll Cardiol 2008 Apr 22;1(16):1564-72.

70) McCullough PA, Ahmed AB, Zughaib MT, Glanz ED, Di Loreto MJ. Treatment of hypertriglyceridemia with fibric acid derivatives impact on lipid subfractions and translation into a reduction in cardiovascular events. Rev Cardiovasc Med 2011; 12: 173-85.

71) Patel SS, Goyal RK, Shah RS, Tirgar PR, Jadhav PD. Experimental study on effect of hydroalcoholic extract of *Emblica Officinalis* fruits on glucose homeostasis and metabolic parameters. Ayu 2013; 34(4): 440-44.

72) Kumar K, Bhowmik D, Dutta A, PdYadav A, Paswan S, Srivastava S, Deb L. Recent trends in potential traditional Indian herbs Emblica Officinalis and its medicinal importance. Journal of Pharmacognosy and Phytochemistry 2012 May;1(1).

73) Patil BS. Effect of Terminalia Arjuna and Emblica Officinalis extract on cardiovascular system in albino wister rats. 2017 PhD thesis; BLDE University,Bijapur.

74) Lama A, Saika H. Effects of Emblica Officinalis(Amla) on serum lipids and Atherogenesis in albino rats fed with high fat diet. Indian Medical Gazette 2013July:271-75.

75) Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant activity of tannoid principles of *Emblica Officinalis* (amla) in chronic stress induced changes in rat brain. Indian J Exp Biol. 200 Sep; 38(9):877-80.

76) Neelima N, Sudhakar M, Lakshmi BVS. Antistress activity of ethanolic extract of Emblica Officinalis fruits in stress induced biochemical and physiological perturbations. International Journal of Research in Pharmacology and Pharmaceuticals 2014 Jan-March; 3(10): 72-79.

77) Gopa B, Bhatt J, Hemvanti KG. A comparative clinical study of hypolipidemic efficacy of Amla (Emblica Officinalis) with 3-hydroxy-3 methylglutaryl coenzyme-A reductase inhibitor Simvastatin. Indian Journal of Pharmacology 2012; 44(2):238-42.

78) L Anila, NR Vijayalakshmi. Flavonoids from *Emblica Officinalis* and *Magnifera Indica*

Effectiveness for dyslipidemia. J Ethnopharmacol 2002;79(1):81-7.

79) Mann S, Beedie C, Jimenez A. Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendation. Sports Medicine 2014; 44(2):211-21.

80) Patel SS, Goyal RK. Prevention of diabetes induced myocardial dysfunction in rats using the juice of the Emblica Officinalis fruit. Experimental & Clinical Cardiology 2011;16(3):87-91.

81) Roy A, Kundu D, Mandal T, Bandopadhyay U, Ghosh E, Ray D. A comparative study of heart rate variability tests and lipid profile in healthy young adult males and females. Nigerian Journal of Clinical Practice 2013;16(4):424-28.

82) Chakroborti D, Verma R. Amleiorative effect of Emblica Officinalis aqueous extract on orchratoxin induced lipid Peroxidation on the kidney and liver of mice. International Journal of Occupational medicine and Environment Health 2010;23(1):63.

83) Sultana S, Ahmed S, Khan N, Jahangir T. Effects of *Emblica Officinalis*(Gaertn) on CCl4-induced hepatic toxicity and DNA synthesis of wister rats. Indian J Exp Biol 2005; 43:430-6.

84) Kaur J, Kaur D, Singh H, Khan MU. Emblica Officinalis:a meritocratic drug for treating various disorders. Indo American Journal of Pharmaceutical Research 2013;3(6).

85) Naampoothiri SV, Prathapan A, Cherian OL, Raghu KG, Venugopalan VV, Sundaresan A. In vitro antioxidant and inhibitory potential of Terminalia bellerica and Emblica Officinalis fruits against LDL oxidation and key enzymes linked to type 2 diabetes. Food and chemical Toxicol 2011; 49(1):125-31.

86) Das KK, Chadchan KS, Reddy RC, Biradar MS, Kanthe PS, Patil BS, Ambekar JG, Bagoji IB. effects of som e indigenous plants of North Karnataka(India) on cardiovascular and glucose regulatory systems in diabetes rats. Cardiovasc hematol Agents Med Chem 2017;

87) Chang WC, Wu JS, Chen CW, Kuo PL, Chien HM, Wang YT, et al. Protective effect of vanillic acid against hyperinsulinemia, hyperglycemia and hyperlipidemia via alleviating hepatic insulin resistance and inflammation in high fat diet fed rats. Nutrients 2015; 7(12):9946-59.

88) Walia K, Boolchandani R, Dhand S, Antony B. improving glycaemic and lipidemic profile with amla powder (Emblica officinlais) supplementation in adults with type 2 diabetes mellitus. IJBAMS 2015;5(2):251-58.

89) Singh MK, Dwivedi S. Arsenic induced hepatic toxicity and its attenuation by fruit extract of embllica Officinalis(Amla) in mice. Ind J Clin Biochem 2014;29(1):29-37.

90) Reddy DV, Padmawati P, Gopi S, Paramahamsa M, Varadacharyulu. Protective effect of Emblica Officinalis against alcohol induced hepatic injury by ameliorating oxidative stress in rats. Ind J Clin Biochem 2010; 25(4):419-24.

CHAPTER 3

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES OF THE STUDY

Aim:

To assess the effect of Ethanolic extract of *Emblica Officinalis* on intermediary metabolism of Albino Wister rats fed with high fat diet.

Objectives:

To evaluate the effect of Ethanolic extract of *Emblica officinalis* (Amla) in Albino rats treated sub chronically with high fat diet.

- Evaluation markers are:

a. Gravimetry

b. Electrophysiology

c. Hematology

d. Biochemistry: a) lipid profile b) Liver function test (LFT), c) Kidney function test (KFT) d) Glucose homeostasis e) Oxidative stress parameters f) Nitric Oxide (NOx)

e. Molecular marker: Endothelial nitric oxide synthase (NOS3)

f. Histopathology (Myocardium, Elastic artery, Liver and Kidney).

Hypothesis:

It may be hypothesized that *Emblica officinalis* (Amla) protects against high dietary fat induced alterations of intermediary metabolic functions of albino rats.

CHAPTER 4

MATERIAL AND METHODS

MATERIALS AND METHODS:

4.1: Experimental animals:

Albino Wistar rats weighing 180 to 230gms were obtained from animal house of Shri B M Patil Medical college Hospital & Research Centre, BLDE University, Vijayapura. All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC; ref. No. 664/15, dated 7/12/2015)) of Shri B M Patil Medical college Hospital & Research Centre, Vijayapur.

An experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), INDIA[1].

All the five group animals were acclimatized for 7 days to the laboratory conditions at 22-24^{0}C and maintained 12 hour. Light/dark cycle.

Sample size: Sample size has been calculated using power analysis[2].

$$n = \frac{(Z\alpha + Z\beta)^2 \, X2XSD^2}{d^2}$$

Where $Z\alpha$ – Level of significance =1%

$Z\beta$ - Power of the study =90%

SD – Common standard deviation of two groups.

d= Difference between means. (Effect size)

So 6 no of rats in each group like 30 rats were in each set of experiment.

Authentication of drugs: Fruits of *Emblica Officinalis* (Amla) were identified and authenticated by Department of Botany, KCP Science College, Vijayapur and Voucher Specimen No. BMPP/03 is deposited in our research laboratory for further reference.

Extraction of drugs:

Received permission to obtain ethanolic extraction of *Emblica officinalis* (Amla) drugs from the Department of Pharmacology, BLDEA College of Pharmacy, Vijayapura. Fruits of *Emblica officinalis* were allowed to dry and dried fruits were coarsely powdered. Four hundred and eighty gram of the dried powdered fruit material was extracted with 99% ethanol using Soxhelet apparatus at a temperature below 60°C for 24 hours. The solvent was evaporated under vacuum which was collected as semisolid mass with (percentage yield 26%) with respect to the dried powder. This extract was stored as stock solution in refrigerator and diluted with distilled water when required[3].

Extract yield was calculated by using formula,

% yield of extract = w1/w2x100

Where, W1= Net weight of powder in grams after extraction and W2= Total weight of fruit powder in grams taken for extraction[4].

Phytochemical screening:

Different extracts were screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate by using standard protocols[5].

Phytochemical investigations:

Chemical and reagents**:**

Laboratory grade chemicals were used for routine work. Analytical grade reagents (A.R.) were used for analytical work

Absorbents:

Silica gel GF254 (RFCL Ltd Renkem production, New Delhi) was used for TLC

Preliminary Phytochemical studies:

The crude extract obtained by extraction from ethanolic extraction was subjected phytochemical studies

A. Detection of Carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of Carbohydrates

1. Molisch"s test: To 1 ml of test solution added a few drops of 1 % alpha-napthol and 2-3 ml concentrated sulfuric acid along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test.
2. Benedict's test: Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.
3. Fehlings test: Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's(A+B) solution, shooked and heated on a water bath for 10 minutes. The brick red precipitate formed confirmed the test

B. Detection of fixed oils & fats

1. Stain Test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

C. Detection of phytosterols

1. Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
2. Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was

added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

D. Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

1. Modified Borntrager's Test: Extracts were treated with a Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

E. Detection of flavonoids

1. Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
2. Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.
3. Shinoda Test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids. Alcoholic solution of extracts, a pinch of Zinc dust and Conc. HCl was added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

F. Detection of alkaloids

Approximately 50 mg of extract was dissolved in 5 ml of distilled water. Further 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was

tested for the presence of alkaloids as detailed below. Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

1. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.
2. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide) Formation of brown/reddish brown precipitate indicates the presence of alkaloids.
3. Dragendorff's Test: To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.
4. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

G. Detection of saponins

1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
2. Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.
3. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well. The formation of a soluble emulsion confirmed the test

H. Detection of resins

1. Acetone-water Test: Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

I. Detection of phenols

1. Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

J. Detection of tannins

1. Ferric chloride Test: Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. Formation of blue colour indicated the presence of hydrolysable tannins.

2. Gelatin Test: Added five drops of 1% gelatin containing 10% sodium chloride to 1 ml of the test solution. Formation of white precipitates confirmed the test.

4.2: Study design:

TABLE 4.1: Diet preparation[6]:

Diet Source	Ingredients	Control Diet (CD)	High Fat Diet (HFD)
Carbohydrate	Amylum	60%	50%
Protein	Casein	18%	18%
Fat	Vegetable oil	20 %	30 %
Salt and vitamins	NaCl and Multivitamins	2%	2%

Each rat of all groups was fed with control and high fat diet respectively per day on an average 50 gms. We noticed that few of rats were consumed more than 45 gms and few of them consumed less than 40 gms of diet. Every day leftover food was discarded and fresh 50 gms of food was served [7].

4.3: Experimental protocol:

All rats were divided into following five groups with 6 rats in each group. Group 1served as control group fed with control diet, group 2 rats fed with control diet with ethanolic extract of *Emblica Officinalis* (EEO) , group 3 rats were fed with high fat diet, group 4 rats were fed with high fat diet and ethanolic extract of *Emblica Officinalis* (EEO) and group 5 rats were fed with high fat diet and atorvastatin . Dose of EEO was 100mg/Kg b. wt of rats for 21 days[8] and dose atorvastatin was 4mg/kg b.wt of rats[9].

TABLE 4.2: Study protocol:

Sr.NO	Groups		No of rats	Doses to be introduced	Days of treatment
1	Group 1	Control	6/Set	0.9% NaCl	21days(Control diet)
2	Group 2	+ *Emblica Officinalis*	6/set	100mg/Kg	21days (Control diet + EEO supplement)
3	Group 3	Hyperlipidemic	6/set	0.9 %NaCl	21 days (hyperlipidemic diet)
4	Group 4	Hyperlipidemic+*Emblica Officinalis*	6/set	+100mg/Kg	21days (Hyperlipidemic diet+ EEO supplement)
5	Group 5	Hyperlipidemic+ Atrovastatin	6/set	4mg/kg/day	21days (Hyperlipidemic diet+ drug treatment)

4.4: Gravimetry:

The body weight of all rats were recorded in the initial step of experiment on first day and on the last day (22^{nd} day) by using digital weighing machine (Practum1102-10IN). Also percent changes of weight gain of all rats were calculated by using formula,

$$\% \text{ body weight gain} = \frac{\text{Final b.wt} - \text{initial b.wt}}{\text{Final b.wt}} \times 100$$

4.5: Electrophysiology:

Heart rate variability analysis

All rats of each group were anesthetized with an intra-peritoneal injection of 60 mg/kg ketamine and 6 mg/kg xylazine. In dorsal recumbency ECG was recorded on day1 and at the end of 21 days on live animals using BioPac instrument (BioPac MP 100: PC windows based animal

electrophysiology system). All the parameters were analyzed by Biopac Student Lab 4.1 software.

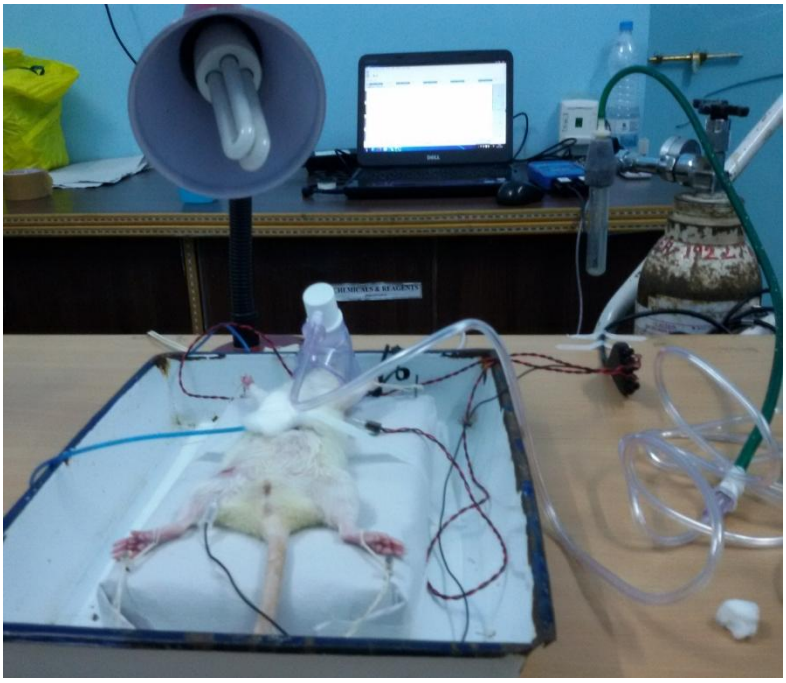


Figure 4.1: ECG recording with BioPac MP 100; Courtesy laboratory of vascular physiology and medicine

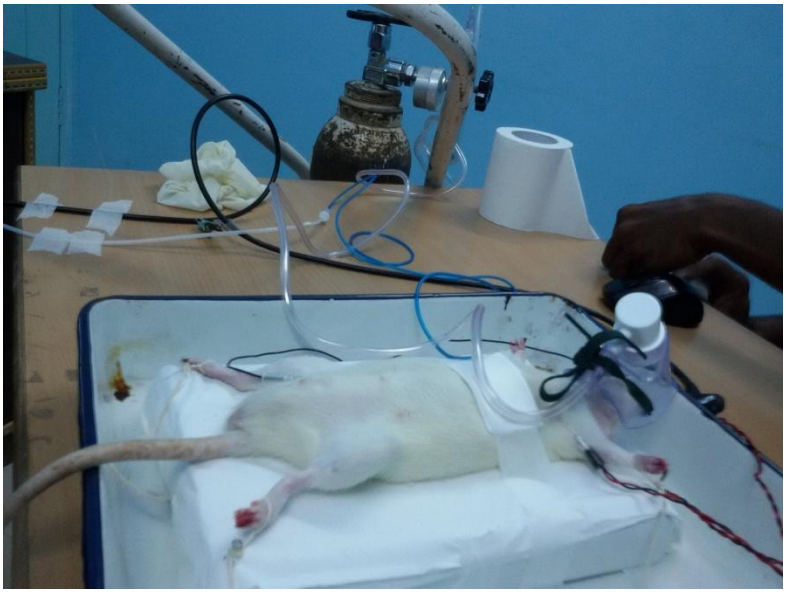


Figure 4.2: ECG recording with BioPac MP 100; Courtesy laboratory of vascular physiology and medicine

4.6: Sample collection:

All animals were kept for an overnight fasting on 21st day. On 22nd day blood was collected in 10% Ethylene Diamine Tetra Acetic Acid (EDTA) tubes by retro orbital puncture using local anesthesia before sacrificing animal. Blood samples were centrifuged at x 900 g for 10 mins and serum was separated.

4.7: Cervical dislocation method:

1) Restrain the rat in a standing position on a firm, flat surface and grasp the base of the tail firmly with one hand. It makes easy to perform the procedure on a surface that the animal can grip and gain access to the base of the skull because rodents often stretch themselves forward when held by the tail.

2) Place the first finger and thumb of the other hand against the back of the neck at the base of the skull.

3) To produce the dislocation, quickly push forward and down with the hand or object restraining the head while pulling backward with the hand holding the tail base.

4) The degree of dislocation can be assessed by feeling for a separation of cervical tissues. The gap of 2-4 mm space will be palpable between the occipital condyles and the first cervical vertebra. Occasionally, the dislocation will be between thoracic vertebrae.

5) Check to confirm respiratory arrest and verify by palpating there is no heart beat[10].

4.8: Haematology:

Haematological parameters were analyzed by XS-1000i closed tube sampling Cysmax automated analyzer (Calibrated by CYSMAX K4500 of Transatia).

Principle:

RBC/PLT: sheath flow direct current on XE and XT series. WBC-semiconductor laser flow cytometry, HGB-clorometric method (SLS).

Testing parameters CBC and S-Part differential 21 parameters like Hb% RBC WBC Platelet PCV & MCHC were analyzed[11].

4.9: LIPID PROFILE:

Serum triglycerides (TG), serum total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein and very low density lipoprotein (VLDL)

i) Serum triglyceride:

Principles: enzymatic analysis of triglycerides was done according to the following equations[12]

Triglyceride + H_2O ⟶ glycerol + fatty acids

Glycerol + ATP ⟶ glycerol-3-phospate + ADP

Glycerol-3-PO4+O_2 ⟶ dihydroxy acetone phosphate+ H_2O_2

H_2O_2 + 4 ⟶ aminoantipyrine + p-chlorophenol- red qunoneimine

Reagent composition:

Pipes buffer, pH 7-0.50 mmol/l

p-chlorophenol- 5.3 mmol/l

potassium ferrocyanate- 10 mmol/l

Magnesium salt -17 mmol/l

4-aminoantipyrine -0.9 mmol/l

ATP- 3.15 mmol/l

Lipoprotein lipase >1800 U/l

Glycerol kinase> 450 U /l

Glycerol-3- phosphate oxidase > 3500 U/l

Peroxidase > 450 U/l

Triglyceride standard solution- 200 mg/dl

The reagent was kept stable for 18 months at 2-8°C. Animal serum was used as the sample. 10 µl of reagent, incubated, for 5 min at 37° C and estimated at 630 nm using biochemical analyzer.

$$\text{Triglyceride (mg/dl)} = \frac{\text{absorbance of sample x 200}}{\text{Absorbance of standard}}$$

ii) Total cholesterol:

Principle: enzymatic estimation was performed according to following equation

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{4 Cholesten- 3- one} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{4- aminoantipyrene} \rightarrow \text{Red quinine} + 4H_2O$$

Cholesterol reagent

Pipes buffer, pH 6.7- 50 mmol/l

Phenol- 24 mmol/l

Sodium cholate- 0.5 mmol/l

4- aminoantipyrene- 0.5 mmol/l

Cholesterol esterase > 180U/l

Cholesterol oxidase > 200U/l

Peroxidase > 1000U/l

Cholesterol standard solution 200 mg/dl

The reagent is stable for 18 months when stored at 2-8° C. animal serum was used as the sample. 10 µl of serum was mixed with 1000 µl of reagent, incubated for 5 min at 37° C and estimated at 630 nm using a biochemical analyzer.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of sample x 200}}{\text{Absorbance of standard}}$$

iii) HDL:

The chylomicrons, VLDL and LDL of serum were precipitated by phosphotungstic acid and magnesium ions. After centrifugation, HDL in the supernatant solution was measured by enzymatic method[13].

HDL cholesterol reagent

Phosphotungstate- 14 mmol/l

Magnesium chloride- 1 mmol/l

Preservative

HDL cholesterol standard- 50 mg/dl.

300 µl of serum was mixed with 300µl of HDL reagent, allowed to stand for 10 min at room temperature, mixed again and centrifuged for 10 min at 4000 rpm. After centrifugation the clear supernatant was separated from the precipitate within 1hr and HDL was determined using cholesterol reagent. 50 µl of supernatant was mixed with 1000 µl of cholesterol reagent, incubated for 5 min at 37 ° C and estimated at 630nm using a biochemical analyzer.

Calculation

$$\text{HDL cholesterol conc. (mg/dl)} = \frac{\text{Absorbance of sample x N x 2}}{\text{Absorbance of standard}}$$

N= Standard concentration (50 mg/dl)

iv) LDL:

$$\text{LDL mg/dl} = \frac{\text{Total cholesterol} - \text{HDL cholesterol- TG}^{14}}{5}$$

$$\text{v) VLDL} = \frac{\text{Triglycerides}^{14}}{5}$$

4.10: Liver Functions Tests:

Levels of SGOT, SGPT, bilirubin, ALP, albumin, A/G ratio and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an autoanalyser (Chemistry analyser CA 2005, B4B diagnostic division, China)

i) Estimation of Albumin:

Principle: the reaction between albumin in serum or plasma and the dye bromoceresol green produces a change in color, which is proportional to albumin concentration[15].

Reagent composition:

Albumin reagent: Succinate buffer (pH-4.2) 75 mmol/l, bromoceresol green 0.14g/l

Albumin standard: Albumin standard concentration 3 g/dl. The reagent is stable for 18 months when stored at 2-8° C. 10 µl of serum was mixed with 1000 µl of reagent, mixed and incubated for 1 minute. The absorbance was read against blank at 630 nm.

Calculation

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of sample x C}}{\text{Absorbance of standard}}$$

Where,
C= concentration of albumin in standard albumin solution (gm/dl)

ii) Total protein estimation:

Principle: calorimetric determinations of total protein based on the principle of the Biuret reaction (copper salt in alkaline medium). Protein in serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of blue color is proportional to the protein concentration[16].

Normal Range: 6.2- 8.0gm/dL

Reagent composition:

Potassium iodide- 6 mmol/l, Potassium sodium tartrate- 21 mmol/l, Copper sulphate- 6 mmol/l, Sodium hydroxide- 58 mmol/l, total Protein Standard- 6 g/dl.

Procedure:

TABLE 4.3: Total protein estimation

	Blank	standard	sample
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	20 µl	-
Sample	-	-	20 µl

The mixture was incubated at 37^{o}C for 15 minutes and the absorbance was measured at 546 nm using a biochemical analyzer.

Conclusion:

$$\text{Total protein concentration (gm/dl)} = \frac{\text{Absorbance of sample x C}}{\text{Absorbance of standard}}$$

iii) Total bilirubin:

Principle: Direct bilirubin in the sample reacts with diazotised sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both direct and indirect bilirubin couple diazo in the presence of cetrimide[17]. The terms direct and total refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing reagents. The direct and indirect bilirubin is approximately equivalent to the conjugated and unconjugated fractions.

Composition (Bilirubin)

Reagent A: Sulfanilic acid 29 mmol/l, Hydrochloric acid 0.2 mol/l, Cetrimide 50 mmol/l

Reagent B: Sodium nitrite- 11.6 mmol/l. Stored at 15-30° C. Reagents were stable until the expiry date shown on the label when stored tightly closed and if contaminations was avoided

during use. Presence of particulate matter, turbidity, absorbance over 0.05 at 540nm, indicate deterioration.

Working reagent: 1 ml of reagent B and 4 ml of reagent A. This was Stable for 20 days at 2-8° C

TABLE 4.4: Standard operating procedure for estimation of total bilirubin

Particulars	Reagent blank	Sample blank	Sample	Standard
Distilled water	100 µl	---	----	----
Sample	----	100 µl	100 µl	----
Standard	----	----	----	100 µl
Reagent A	-----	1000 µl	----	----
Working reagent	1000µl	----	1000 µl	1000 µl

Mixed thoroughly and was allowed to stand for 2 min at room temperature. Absorbance of Sample Blank was read at 540nm against distilled water and absorbance of Sample was measured at 540nm against reagent blank.

Calculations:

$$\text{Bilirubin content in the sample} = \frac{\text{A (sample)} - \text{A(sample blank)}}{\text{A (standard)}} \text{ X C (standard)}$$

Mass concentration (mg/dl) x 17.1 = Substance concentration in µmol/l.

iv) Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)

By IFCC Method

The SGOT test measures the amount of a substance called glutamic-oxaloacetic transaminase (GOT) in the blood. It is an enzyme found in the liver, muscles, heart and red blood cells. It is leaked into the blood from cells which are damaged. This enzyme is also known as aspirate aminotranskinase , aspartate transaminase (AST) [18].

Cliniquant-FSR SGOT reagent, Meril diagnostic, IFCC kinetic method

Principle:

$$\text{L- aspartate + 2 oxaloacetate} \xrightarrow{\text{AST}} \text{oxaloacetate+ L-Glutamate}$$

$$\text{Oxaloacetate + NADH} \xrightarrow{\text{MDH}} \text{Malate + NAD}$$

$$\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L-acetate + NAD}$$

Composition:

Tris Buffer 80 nmol/L pH 7.8, L-aspartate 200 mmol/L, 2- oxaloacetate 12 mmol/L, NADH 0.18 mmol/L, MDH ≥ 600 U/L, LDH ≥ 900UI/L and stabilizers

Materials required:

Laboratory instrumentation, spectro photometer, UV/Vis with thermostatic cuvette holder or clinical analyzer; semi auto or fully automated analyzer, track, heating bath, controls, saline

Test procedure

Dispense in tube: working reagent- 1000 µl, add 50 µl samples, perform other 3 readings at interval of 60 seconds. Calculate the delta A/minute. Mix and incubate for 60 seconds at 30° C, record the absorbance reading.

Result and calculations:

$$\text{Activity in U/L} = \frac{\text{delta A}}{\text{min}} \text{ X } 3376$$

SI conversation factor: 1 U/L x 0.017=1µ kat/L

SGOT activity was expressed in U/L.

v) Estimation of Serum Glutamate Pyruvate Transaminase (SGPT/ ALT)

by IFCC kinetic method[19].

Cliniquant-FSR SGOT reagent, Meril diagnostic, IFCC kinetic method.

Principle:

$$\text{L- aspartate + 2 oxalo} \xrightarrow{\textbf{ALT}} \text{oxaloacetate+ L-Glutamate}$$

$$\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{L-acetate + NAD}$$

Composition: Tris buffer 80 mmol/L pH 7.5, L- alanine500 mmol/L,2-oxoglutarate 12 mmol/L, NADH 0.18 mmol/L, MDH ≥600U/L, LDH ≥ 2000U/L and stabilizers.

Materials: Laboratory instrumentation, spectro photometer, UV/Vis with thermostatic cuvette holder or clinical analyzer; semi auto or fully automated analyzer, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath, controls, saline.

Test procedure:

Add working reagent-1000 µl and sample 50 µl, mix and incubate for 60 seconds intervals.

Calculate the delta A/minute.

Result and calculations:

$$\text{Activity in U/L} = \frac{\text{delta A}}{\text{min}} \times 3376$$

SI conversation factor: 1 U/L x 0.017=1µ kat/L

SGPT activity was expressed in U/L.

Vi) Estimation of Alkaline phosphatase:

Principle: Alkaline Phosphatase catalyses in alkaline medium the transfer of phosphate group from 4-nitrophenyl phosphate to 2-amino-2-methyl-1-propanol, liberating 4-nitrophenol. The

catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405nm[20].

The enzymatic relation is shown as follows:

4- Nitrophenyl phosphate + H_2O ALP Phosphate + 4- Nitrophenol

Required reagents:

Reagent A: 2- Amino-2- methyl- 1- propanol 0.4 mol/l**,** Zinc sulphate 1.2 mmol/l**,** N hydroxy ethylene diamine tri aceticacid 2.5 mmol/l**,** Magnesium acetate 2.5 mmol/l, pH 10.4.

Reagent B: 4- Nitrophenyl phosphate 60 mmol/l.

Working reagent: 4 parts of reagent A is mixed with 1 part of reagent B

The combined reagent is stable for 2 months at 2-8° C. Animal serum was used as the sample 20 µl of serum was mixed with 1000 µl of mixed reagent and estimated in kinetic mode using a biochemical analyzer.

Calculations

$$\text{ALP concentration (U/l)} = \frac{\text{delta A/min X Vt X } 10^6}{\text{E X L X Vs}}$$

Molar absorbance (E) of NADH at 405nm is 18450

L- Light path 1cm

Vt - Total reaction volume is 1.02 at 37° C

Vs - Sample volume is 0.02 at 37° C

4.11: Kidney Function Tests:

Estimations of blood urea and serum creatinine were done [21, 22]

i) Estimation of blood urea*:* by using Bio System Kit

Principle of the method: urea in the sample consumes by means of the coupled reactions described below, NADH that can be measured by spectrophotometry.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4 + CO_2$$

$$NH_4 + \text{NADH} + H^- + \text{2-oxoglucotarate} \xrightarrow{\text{Glutamate dehydogenase}} \text{Glutamate} + NAD^+$$

Composition:

A) Reagent: Tris 100mmol/L, 2-oxoglutarate 5.6.mmol/l, urease >140U/ml, glutamate dehydrogenase > 140U/ml, ethyleneglycol 220 g/l, sodium azide 0.9g/l, pH 8

B) Reagent: NADH 1.5 mmol, sodium azide 9.5 g/l

C) Glucose/Urea/creatinine standard: Glucose: 100mg/dl, urea 50 mg/dl, creatinine 2mg/dl. Aqueous primary standard.

Reagent preparations:

Working reagent: transfer the contents of one reagent B vial into reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4ml reagent A + 1ml Reagent B.

Procedure:

1) Bring the working reagent and the photometer to 37◦c.

2) Pipette into a cuvette

TABLE 4.5: Standard operating procedure for estimation of blood urea

Working reagent	1. 5ml
Standard	10µl

3) Mix and insert the cuvette into the photometer. Start stopwatch.

4) Record the absorbance at 340 nm after 30 sec (A_1) and after 90 seconds (A_2)

Calculations:

Urea concentration in the sample is calculated using following formula

$$\frac{(A_1\text{-}A_2)\ \text{Sample}}{(A_1\text{-}A_2)\ \text{standard}}\ \text{X C standard X sample dilution factor} = \text{C sample}$$

If the urea standard provided has been used to calibrate

TABLE 4.6 Calibration table to estimate blood urea

	Serum and plasma
(A1-A2) sample (A1-A2) Sample	X50= mg/dl X 23.3=mg/dl X8.3 =mmol

ii) Estimation of serum creatinine: by using Bio System Kit

Principle: Creatinine in the sample reacts with picrate in alkaline medium forming a colored complex. The complex formation rate is measured in a short period to avoid interferences.

Composition:

A) Reagent: sodium hydroxide 0.4 mol/L

B) Reagent: picric acid 25mmol/L

C) Glucose/Urea/ creatinine standard: glucose 100 mg/dl, urea 50 mg/dl, creatinine 2mg/dl

Reagent preparation:

Standard is provided ready to use

Working reagent: mix equal volumes of reagent A and reagent B. mix thoroughly. Stable for 1 month at 2-8°c.

Procedure:

1) Bring the working reagent and the photometer at 2-8°c.

2) Pipette into a cuvette

TABLE 4.7: Standard operating Procedure for estimation of serum Creatinine

Working reagent	1 ml
Standard or sample	0.1 ml

3) Mix and insert cuvette into the photometer. Start stopwatch.

4) Record the absorbance at 500nm after 30 seconds (A_1) and after 90 seconds (A_2)

Calculations: creatinine concentration in the sample is calculated using following formula

$$= \frac{(A_1\text{-}A_2) \text{ sample}}{(A_2\text{-}A_1) \text{ standards}} \text{ X C standard X sample dilution factor}$$

If the creatinine standard provided has been used to calibrate

	Serum and plasma
(A1-A2) sample (A1-A2) Sample	X 2= mg/dl X 177=µmol/L

4.12: Oral Glucose Tolerance Test:

OGTT was performed on rats in all groups after an overnight fast on 20th day. All rats were fed with 0.35 g of glucose/100 g of b wt[23]. Blood samples were collected from the tail vein after a small tail incision at the indicated time points (0 hr, 0.5 hr, 1 hr, 1.5 hr and 2 hr) and evaluated these samples with a commercial hand-held Glucometer (ACCU-CHEK Active; Roche).

Fasting insulin was estimated by ELISA technique by using rat insulin kit. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) Index was calculated by using formula = fasting insulin levels X fasting blood sugar level/405[24].

4.13: Estimation of Fasting Insulin

By Chemiluminescence immunoassay (CLIA)[25,26]

Principle: the insulin CLIA Kit is based on a solid phase enzyme linked immunosorbent assay. One anti-insulin antibody get utilizes by the assay for the solid phase immobilization and another anti insulin antibody in the antibody enzyme conjugate solution. The test specimen and standards are added to the insulin antibody coated microliter wells. Then anti- insulin antibody labeled with horseradish peroxidase is added. If insulin present in the specimen, will join with the antibody on the wells and results in insulin molecules sandwiched between the enzymes linked antibodies and solid phase. After incubation of one hour at room temperature, wells are washed with water to remove unbound labeled antibodies. A solution of Chemiluminescent substrate is then added and read relative light units in an illuminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of insulin in the serum. By reference to a series of insulin standards assayed in the same way, the concentration of insulin in the unknown sample is quantified.

Assay procedure:

- Secure the desired number of coated well in the holder. Dispense 50 µl of insulin standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
- Dispense 100 µl enzymes conjugate reagent into each well. Mix well for 30 seconds and incubate at room temperature for 60 minutes.
- Remove the mixture of incubation by emptying the plate content into a waste container. Rinse and empty the micro liter plate 5 times with 1X wash buffer (300 µl each well).

Strike the micro liter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

- Dispense 100 µl of Chemiluminescence substrate reagent into each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
- Real the optical density at 450 nm with a micro liter plate reader within 15 minutes.

4.14: Estimation of Oxidative Stress Markers:

Endogenous oxidative stress markers:

4.14.1: Malondialdehyde (MDA) estimation:

By TBARS method[27]

Introduction: Malondialdehyde is used as a oxidative stress marker in the biological systems. It is formed when PUFA gets degraded by free radical chain reaction. It is one of the end products of lipid Peroxidation. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause oxidative stress.

Principle: MDA formed by the breakdown of poly unsaturated fatty acids (PUFA) serves as a convenient index to determine the extent of lipid Peroxidation. It reacts with TBA to give a pink color which is read at 535 nm.

Reagents required:

Tri chloro acetic acid (TCA)-(CH_3COOC_{13})

2-Thiobarbutric acid (TBA)-($C_4H_4N_2O_2S$)

Hydrochloric acid (HCl)

Malondialdehydebis (dimethyl acetal) –($C_7H_{16}O_4$)

Preparation:

TCA-TBA-HCl reagent:

- 0.25 N HCl: 2.21 ml of conc. HCl ids made upto 100ml with distilled water.
- 15% TCA and 0.375% TBA- 15gm TCA and 0.375 gm of TBA is dissolved in 100ml of 0.25 N HCl. The reaction mixture is warmed to dissolve the contents and stored at 4° C.

MDA standard (stock-16.4 µg/ml)

- 16.4 µl of the standard Melandoaldehyde solution is taken and made up to 100ml with distilled water.

MDA working standard (Working -16.4 µg/ml)

- 100 µl of the stock is made up to 10 ml with distilled water.

Procedure

Standardization: (Range: 2-10 µM/L)

TABLE 4.8: Standard operating procedure for estimation of MDA

Reagents	**Blank**	**1**	**2**	**3**	**4**	**5**
Volume Of MDA(ml)	0.0	0.2	0.4	0.6	0.8	1
Vol. of DW(ml)	1	0.8	0.6	0.6	0.2	-
Conc. Of MDA (µM/L)	0.0	2	4	6	8	10
TBA-TCA-HCl	1	1	1	1	1	1

Boil in water bath for 15 mins. The optical densities are plotted against the concentrations on a graph.

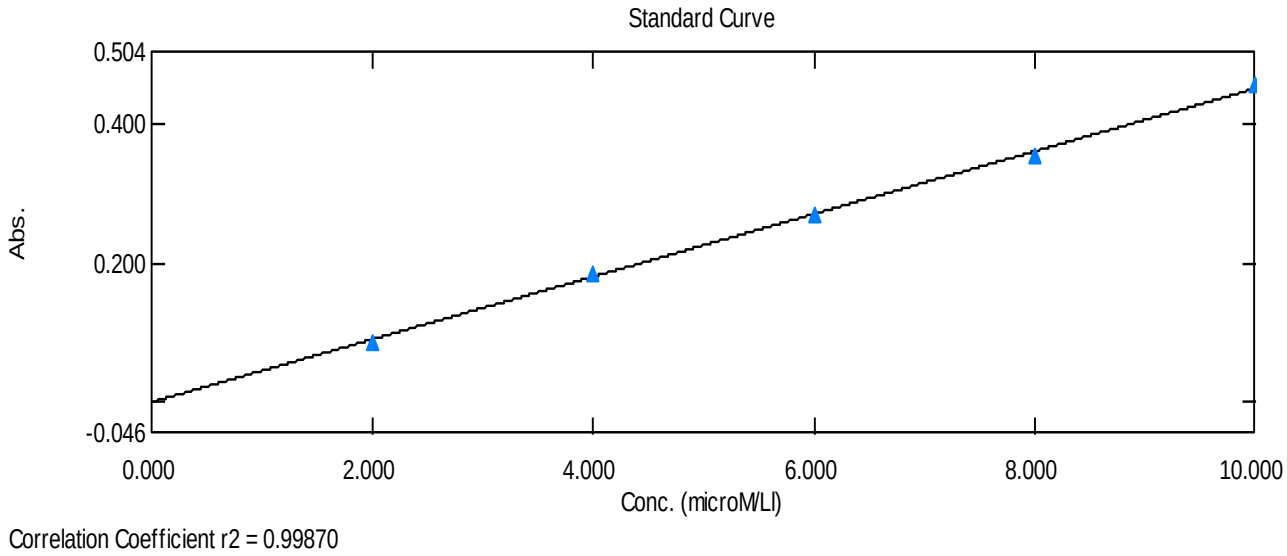


Figure 4.3: Standard graph MDA

Estimation of malondialdehyde in sample:

Sample preparation: 100µl serum is diluted to 500µl with distilled water.

The optical densities of the test samples is directly proportional to the concentration of MDA in the sample and calculated by the plotting against the standard graph and multiplied by the respective dilution factors. The final concentration is expressed as µM/L.

4.14.2: Estimation of superoxide dismutase (SOD):

By Marlund and Marklund method[28]

Principle: superoxide anion is involved in auto- oxidation of pyrogallol at alkaline pH8.5. SOD inhibits the auto oxidation of pyrogallol, which can be determined as an increase in absorbence per two minutes at 420 nm on a spectrophotometer.

Reagents: **1. Tris-EDTA Buffer**

500ml Tris-EDTA buffer was prepared by adding

Tris buffer – 0.0275 gm (50 mM)

EDTA- 0.186 gm of (1mM) and to this

HCl- 50 mM

Adjust the pH 8.5 and volume was made up to 500ml.

2. Pyrogallol(20 mM): 25 mg pyrogallol was dissolved in 10 ml distilled water.

Procedure: two test tubes were taken and labeled as test (T) and controls (C), addition of reagents in ml, were prepared as per the following protocol.

SOD ASSAY

TABLE 4.9: Standard operating procedure for estimation of SOD

Reagents	T	C
Tris buffer	2.85ml	2.9ml
Hemolysate	0.05ml	-
Pyrogallol	0.1ml	0.1ml

The contents of tubes were mixed and absorbance was taken at 420 nm exactly after 1 minute 30 seconds and 3min 30 seconds. The absorbance per two minutes was recorded.

Calculations: Difference in absorbance (ΔA/min) was calculated as

$$\Delta A/min = \frac{\text{OD at 3 min 30 secs-OD at 1 min 30 sec}}{2}$$

$$\text{Serum SOD calculations} = \frac{\Delta A/min \text{ of control} - \Delta A/min \text{ of test}}{\Delta A/min \text{ of control} \times 50} \times 100 \times \frac{1}{\text{volume of serum}}$$

$$\text{SOD U/ml of hemolysate} = \frac{C-T}{Cx50} \times 100 \times \frac{1}{0.1}$$

$$= \frac{C-T}{C \times 50} \times 1000$$

$$= \text{——} \text{ U/ml}$$

One unit of SOD is defined as the amount of enzyme required to cause 50 % inhibition of pyrogallol auto-oxidation.

4.14.3: Estimation of reduced glutathione (GSH):

By Earnest Beutler method[29],

Principle: non- protein groups of red blood cells (RBC) are present in the form reduced glutathione. 5,5' dithiobis -2- nitrobenzonic acid (DTNB) is a disulphide compound which is really reduced by sulphydryl compounds, forming a highly colored yellow compound. Optical density was measured at 412 nm and it is directly proportional to GSH concentration.

Reagents:

1) Precipitating solution = 1.67 gms of glacial metaphosphoric acid, 0.2 gm of disodium or dipotassium ethylene diamine tetra acetic acid(EDTA) and 30gm of sodium chloride was dissolved in 100 ml of distilled water.

2) Phosphate solution= 0.3M Na_2HPO_4 (di-sodium hydrogen phosphate) was prepared by dissolving 4.68 gms in 100 ml distilled water.

3) 1% sodium citrate= 1 gm of sodium citrate was dissolved in 100ml distilled water.

4) DTNB reagents= 40 mg 5,5' dithiobis -2- nitrobenzonic acid (DTNB) was dissolved in 100 ml of 1 % sodium citrate.

5) Reduced glutathione standard (0.5 mg/ml) = Take 5 mg of reduced glutathione and dissolved in 10 ml of distilled water.

Procedure: Three test tubes were taken and labeled as blank, standard and test. The procedure for GSH assay is given as below,

TABLE 4.10: Standard operating procedure for estimation of GSH

Reagents	**Blank**	**Standard**	**Test**
Whole blood	----	-----	0.2 ml
Standard	---	0.4 ml	----
Distilled water	2 ml	1.6 ml	1.8 ml
Mixed well			
Precipitating solution	3ml	3ml	3ml
Keep for 5 mins, centrifuged and 1ml supernatant was added in a separate labeled test tubes			
Phosphate solutions	4ml	4ml	4ml
DTNB reagent	0.5 ml	0.5 ml	0.5ml

Mixed and absorbance was read at 412 nm against the blank within 5 minutes

Calculation:

$$\text{Calculation of erythrocyte reduced glutathione} = \frac{\text{OD of test}}{\text{OD of Std}} \times \frac{\text{conc. of Std}}{\text{Volume of test}} \times 100$$

= ----------mg/ml

4.14.4: Estimation of Catalase:

By A K Sinha Method[30].

Principle: catalase is ubiquitous enzyme that is present in most of aerobic cells. Catalase is involved in the detoxification of hydrogen peroxide, a reactive oxygen species, which is atoxic product of the both normal metabolism and pathogenic ROS production. This enzyme catalyses the conversion of two molecules of hydrogen peroxide to molecular oxygen and two molecules

of water. Catalase demonstrates peroxide activity, in which low molecular weight alcohols can serve as electron donors.

Catalytic activity $2\ H_2O_2 \longrightarrow O_2 + 2H_2O$

Peroxidatic activity $2\ H_2O_2 + AH_2 \longrightarrow A + 2H_2O$

While aliphatic alcohols serve as specific substrates for CAT, other enzymes with peroxisatic activity do not utilize these subtracts.

Reagents:

Phosphate buffer (0.01 M pH 7.4): 283 mg of disodium hydrogen phosphate, 19 mg of potassium dihydrogen phosphate and 800mg of sodium chloride was dissolved in 100ml of distilled water.

Hydrogen peroxide (0.2 M): 56.6ml of hydrogen peroxide was diluted to 100ml with distilled water.

Dichromate acetic acid reagent: 5% of potassium dichromate was made by dissolving 1.25 gm of potassium dichromate in 25 ml of distilled water. The above solution was mixed with glacial acetic acid in the ratio of 1:3.

TABLE 4.11: Standard operating procedure for estimation of Catalase

Concentration (M)	Stock solution (ml)	Distilled water (ml)	Total volume (ml)
0.005	0.05	9.95	10
0.01	0.1	9.9	10
0.02	0.2	9.8	10
0.04	0.4	9.6	10
0.08	0.8	9.2	10
0.1	1	9	10

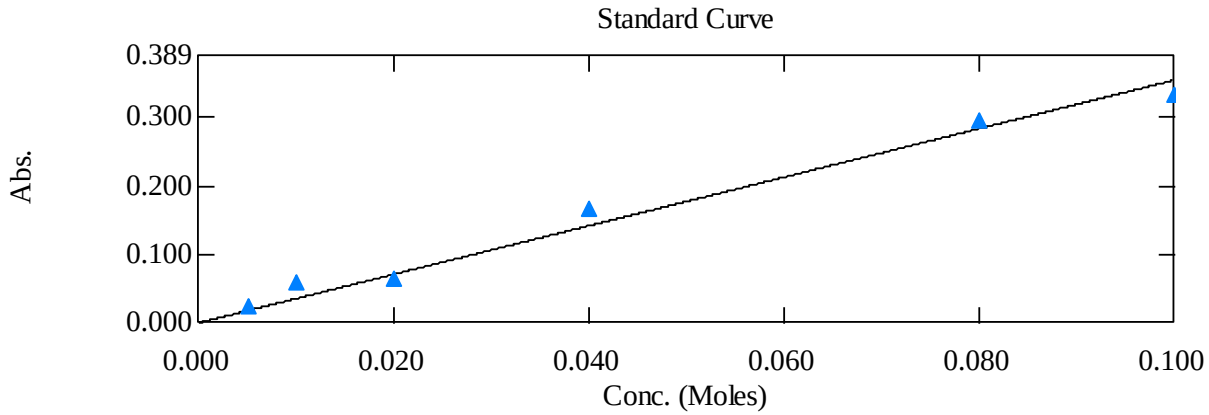


Figure 4.4: Standard graph for Catalase

Addition sequence of catalase standard graph reagents:

To each 1 ml of dilution 4 ml of dichromate- acetic acid reagent was added. Further, the samples were heated in boiling water bath for 15 min. the mixture was allowed to attain room temperature and the absorbance was read at 570 nm against blank (excluding H_2O_2), a standard graph was plotted between absorbance versus moles of H_2O_2 remaining in the solution.

Exogenous oxidative stress

4.14.5: Estimation of Serum vitamin C:

Serum vitamin C was estimated by 2, 4-dinitrophenylhydrazine method (Roe JH et al., 1943; Brewster MA., 1996)[31]

Principle: Ascorbic acid was oxidized by copper to form dehydroascorbic acid and diketogluconic acid. These products were treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This compound in strong sulfuric acid undergoes rearrangement to form a colored product which was measured at 520 nm. The reaction was run in the presence of thiourea to provide a mildly reducing medium which helps to prevent interference from non-ascorbic acid chromogen.

Reagents

1.10 % Trichloroacetic acid: 10 gm of Trichloroacetic acid (TCA) was dissolved in distilled water to prepare a final volume of 100 ml.

2. DTC reagent: 3.0 gm of 2, 4-dinitrophenyl hydrazine (DNPH), 0.4 gm Thiourea and 0.05 gm copper sulphate were added to 9N sulfuric acid. The final volume of 100 ml was prepared.

3. 65 % sulfuric acid: 65 ml of sulfuric acid was dissolved in 35 mL distilled water.

4. Stock standard: 100 mg ascorbic acid was dissolved in 100 mL of 5 % TCA.

5. Working standard (10µg/mL): 1mL of stock standard was dissolved in 100 mL of 5 % TCA.

Procedure

1. Deproteination: 500 µl of sample was mixed with 500 µl of 10% TCA in an eppendorf tube. Vortexed and then centrifuged. The clear supernatant protein free filtrate was used.

2. 500 µl of sample and standards were taken in a test tube separately to which 100 µl DTC reagent was added.

3. Incubated at 37^0C for 3 hours.

4. 750 µl of 65% sulfuric acid was added to all the test tubes.

5. Vortexed and kept for 30 minutes at room temperature.

6. Absorbance was read at 520 nm.

Calculation: Concentration of Serum Vitamin C

$$= \frac{\text{OD of test}}{\text{OD of Std}} \text{ X } \frac{\text{conc. of Std}}{\text{Volume of test}} \text{ X100}$$

$$= \frac{\text{OD of test}}{\text{OD of Std}} \text{ X } \frac{0.005}{0.25} \text{X100}$$

$$= \frac{\text{OD of test}}{\text{OD of Std}} \text{ X } 2$$

= ……. mg/dl

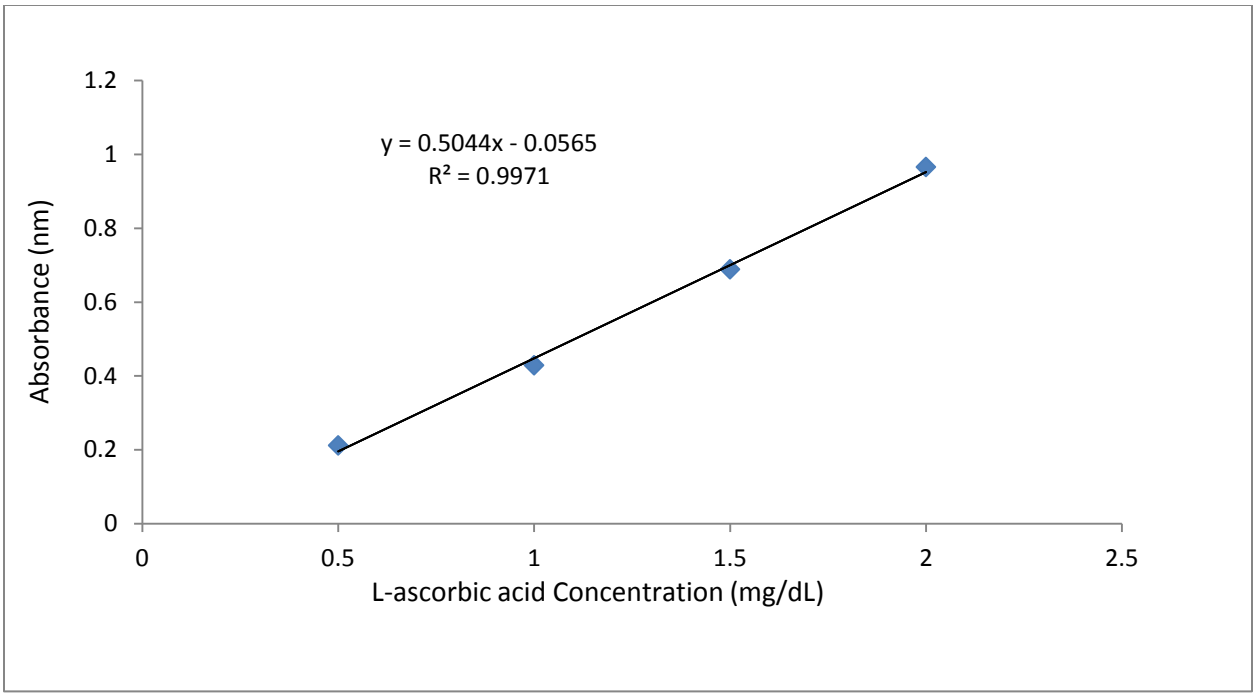


Figure 4.5: Standard graph Estimation of Serum vitamin C

4.14.6: Vitamin E estimation:

By Jameel Jargar and Das Method[32]:

Reagents:

Stock standard of α- tocopherol (0.27% w/v): 270 mg of α- tocopherol acetate dilute in 100ml of absolute ethanol and mix thoroughly.

2,2'-bipyridyl (0.12% w/v): 120 mg 2,2'- bipyridyl dissolved and made volume up to 100ml of n- propanol and kept in brown bottle.

Ferric chloride (0.12 w/v): 120 mg $FeCl_3 6H_2O$ dissolved in 100ml ethanol and kept in brown bottle. All the solutions are stable at room temperature.

Procedure:

Working standard of α- tocopherol (27 µg/mL): Dilute 1ml of stock standard to 100ml absolute ethanol to obtain concentration 27 µg/mL. This solution is stable at room temperature. In six centrifuged tubes labeled as blank, S_1, S_2, S_3, S_4, S_5 place 00,150,300,450,600 and 750 µl of working standard α- tocopherol(27 µg/mL) in respectively and add absolute ethanol to make the

volume of each tube equal as 750 µl. these solutions are equivalent to 4 µg/mL, 8 µg/mL, 12 µg/mL,14 µg/mL, µg/mL and µg/mL of α- tocopherol respectively.

TABLE 4.12: Standard operating procedure for estimation of Vitamin E

S. No	working standard		D W µl	Xylene µl		2-2'bipyridyl µl	$FeCl_3$ µl
	α- tocopherol	Ethanol					
B	0	750	750	750	Centrifuge for10 mins at 3000 rpm.500 µl xyline layer taken out	500	100
S1	150	600	750	750		500	100
S2	300	450	750	750		500	100
S3	450	300	750	750		500	100
S4	600	150	750	750		500	100
S5	750	0	750	750		500	100

After 2 mins read OD at 492 nm.

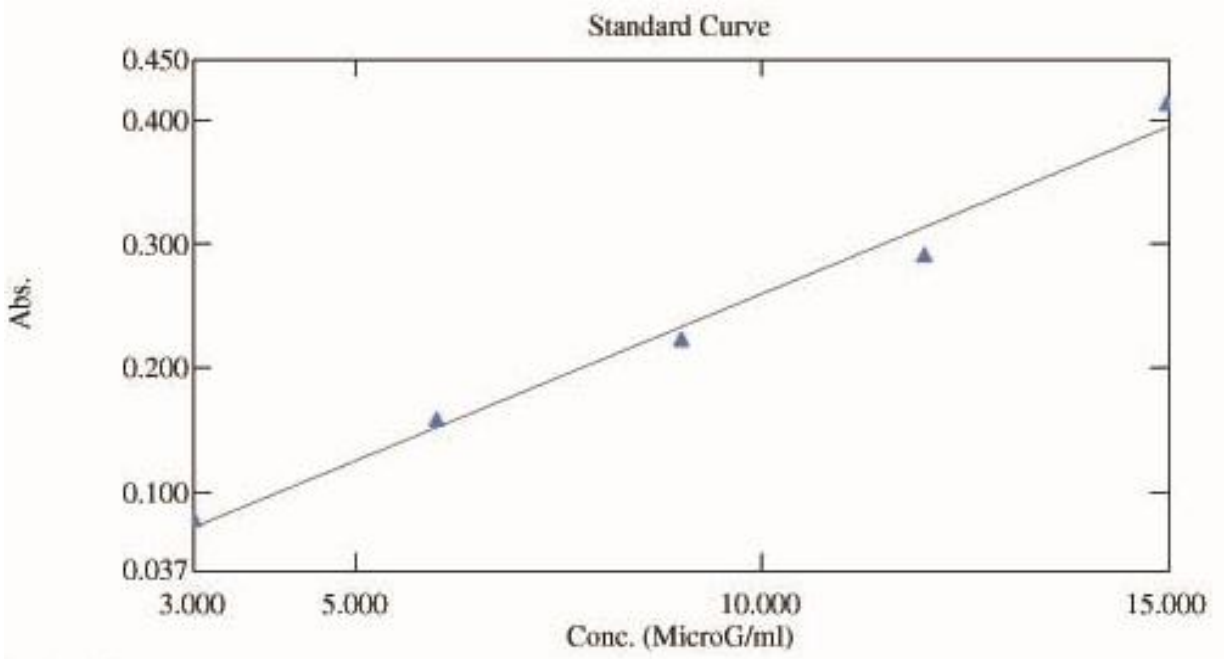


Figure 4.6: Standard graph Vitamin E estimation

Analysis of serum α- tocopherol

Step 1: Allow 3 ml of blood to clot in centrifuge tube for 2 hrs at room temperature and centrifuge at 3000 rpm for 15 mins to get serum.

Step 2: two centrifuge tubes labeled as T (Test) and B(Blank). To the sample tube add 750 µl absolute ethanol and 750 µl serum. Add the serum slowly with shaking to obtain a finely divided protein precipitate. To the blank tube add 750 µl distilled water 750 µl absolute ethanol. Stopper the tubes tightly by wrap paper and shake vigorously for at least 30 sec. to all these tubes add 750 µl xylene. Again stopper the tubes tightly by wrap paper and shake vigorously for at least 30 sec and centrifuge all tubes for 10 min at 3000rpm.

Step 3: transfer 500µl of xylene layer i.e. supernatant into cleaned and well labeled small sized test tubes.

In each tube add 500µl of 2-2'bipyridyl µl and 100 µl ferric chloride solution. After that wait for 2 mins.

Step 4: transfer 200 µl of solution to the microwells respectively, and readings are taken in ELISA (Erba, Transasia), with rapid measure mode. Set primary wavelength as 492 nm and measure the absorbance within 4 mins.

Calculations:

$$\textbf{Conc. of Vit E } \mu g/mL = \frac{\text{OD of test} - \text{OD of Blank}}{\text{Slope}} \text{ X dilution factor}$$

$$\text{Where, slope} = \frac{Y2-y1}{X2-x1}$$

X and y are conc. and absorbance of standards respectively.

4.15: Estimation of Nitrosative stress markers:

4.15.1: Estimation of Nitric Oxide:

By Griess Method: (Kinetic cadmium reduction)[33]

Principle: Nitrate, the stable product of nitric oxide is reduced to nitrite by cadmium reduction method. The nitrite produced is determined by diazotization with sulphanilamide and coupling to N-naphthylethylenediamine. The intensity of colored complex is measured at 540 nm.

Reagents:

1. Cadmium granules (2.5 – 3 gram granules in assay, stored in 0.1M/L H2SO4)

2. Glycine–NaOH buffer (pH 9.7): 7.5 gm of glycine was dissolved in 200ml deionised water, then the pH was adjusted to 9.7 by 2M NaOH and was diluted to 500 ml by deionised

3. Sulfanilamide: 2.5 gm of sulfanilamide was dissolved in 250 ml of warm 3M/L HCl and

allowed to cool.

4. N-naphthylethylenediamine : 50 mg of N-naphthylethylenediamine was dissolved in deionised water and the volume was adjusted to 250 ml.

5. Stock standard sodium nitrite solution (0.1 mol/L): 690 mg sodium nitrite was dissolved in 100 ml of 10 mmol/L of sodium borate solution.

6. Working standard solution (10 µmol/L): 10µl of stock was diluted to 100 ml with 10mmol/L solution of sodium borate.

7. $ZnSO_4$ solution (75 mmol/L)

8. NaOH solution (55 mmol/L)

9. H_2SO_4 solution (0.1 mol/L)

10. $CuSO_4$ solution (5 mmol/L): 125 mg of $CuSO_4$ was dissolved in 100 ml of glycine - NaOH buffer.

Procedure:

Part I: - *Deproteinization*: A centrifuge tube was taken and additions were made as follows

TABLE 4.13: Standard operating procedure for Deprotenization

Serum.	0.5 ml
75 mM $ZnSO_4$	2.0 ml
55 mM NaOH	2.5 ml

Tube was centrifuged at the speed of 1500 rpm for 10 min. and supernatant was collected.

Part II: Activation of Cadmium granules

1. Cadmium granules were stored in 0.1 mol/L H_2SO_4 solution.

2. At the time of assay the acid from granules was rinsed three times with deionized water.

3. Then the granules were swirled in 5 mmol/L $CuSO_4$ solution for 1-2 minutes.

4. These copper coated granules were drained and washed by glycine - NaOH buffer.

5. These activated granules were used within 10 minutes after activation.

6. The granules after use were washed by deionized water and stored in 0.1 mol/L H2SO4 solution. Same procedure for activation was followed each time.

Part III: Nitrite assay a set of three test tubes was arranged as follows and respective additions were made as follows.

TABLE 4.14: Standard operating procedure for estimation of Nitric oxide (NOx)

Reagent	**Test**	**standerd**	**Blank**
Glycine-NaOH Buffer	500 µl.	500 µl.	500 µl.
Supernatant	500 µl.	-	-
Standard 10 µmol/L	-	500 µl.	-
D/W	-	-	500 µl.
Cadmium granules	2	2	2
Cadmium granules were swirled and tubes were kept at RT for 90 min.			
D/W	1.0 ml.	1.0 ml.	1.0 ml.
Content from all this tube was mixed well and diluted solutions were taken in following tubes			
Above diluted solutions	1.0 ml.	1.0 ml.	1.0 ml.
Sulfanilamide	0.5 ml	0.5 ml	0.5 ml
N-napthylethylendiamine	0.5 ml	0.5 ml	0.5 ml

After 20 minutes waiting all tubes were read at 540 nm.

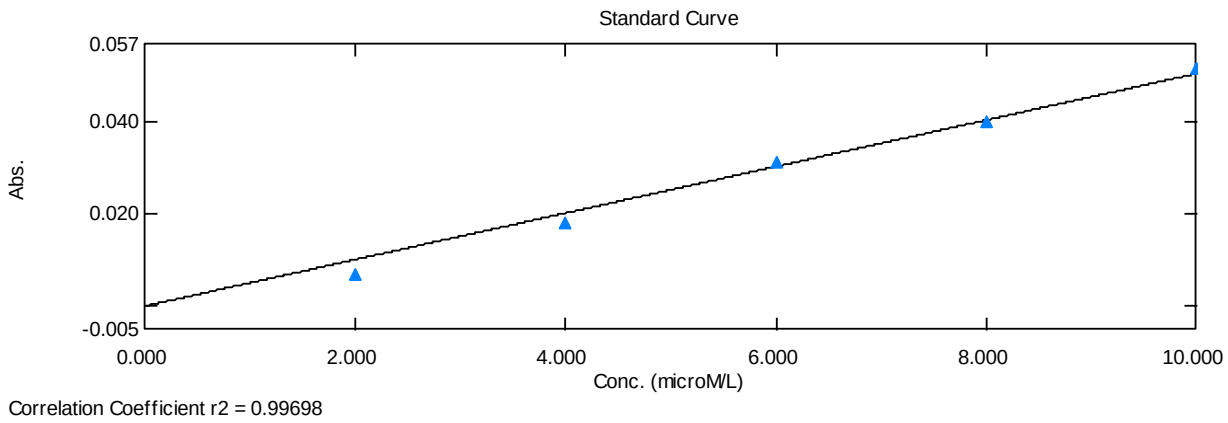


Figure 4.7: Standard graph Estimation of Nitric Oxide

Calculation: Serum Nitrite (µm/L) = (T-B) ÷ (S-B) X 100

4.15.2: Estimation eNOS/NOS$_3$

By ELISA Kit method (YH ELISA Kit)

This kit is used to assay the endothelial nitric oxide synthase (eNOs) in the sample of rat serum, blood plasma.

Principle:

This kit is based on biotin double antibody sandwich technology to assay rat eNOs.

Add eNOs to wells those are precoated with eNOs monoclonal antibody and incubate. After incubation, add eNOs antibodies labeled with biotin to unite streptavidine HRP, which forms the immune complex. Remove unbound enzyme after incubation and washing. Then add substrate A and B. the solution will turn blue and change to yellow with the effect of acid. The shades of solution and concentration of rat eNOs are positively correlated.

Assay procedure:

TABLE 4.15: Standard operating procedure for estimation of eNOS/NOS3

96ng/ml	Standard No 5	120 µl original standard + 120 µl standard diluents
48ng/ml	Standard No 4	120 µl Standard No 5 +120 µl standard diluents
24ng/ml	Standard No 3	120 µl Standard No 4+ 120 µl standard diluents
12ng/ml	Standard No 2	120 µl Standard No 3+ 120 µl standard diluents
6ng/ml	Standard No 1	120 µl Standard No 2+ 120 µl standard diluents

Measure the absorbance (OD) of each well under 450nm with in 10mins. According to OD value of samples, calculate the concentration of the corresponding sample.

4.16: HISTOPATHOLOGY

4.16.1: Fixation of tissues:

The fixation of tissues in 10% of formalin

Dissection and Fixation of Tissues:

Rats were carefully dissected out by taking midline incision after opening the anterior chest wall and abdomen. Heart, elastic artery, Liver and Kidney carefully collected. Then it was weighed immediately and fixed in 10% formalin. After fixation, it was placed in 70% alcohol for 6-8 hours during the day, then in 90% alcohol for overnight. Next day three changes of absolute alcohol were given for one hour each.

The tissue was blotted with blotting paper and placed in xylene for about 30 min for clearing. Then tissue was subjected to 3 changes of paraffin wax at 56^0 c to 60^0c temp for one hour each. Then tissue was embedded. 'L' shaped moulds were smeared with glycerine and fresh filtered wax poured into it to fill it almost. Any air bubbles formed were removed by hot spatula. Then the tissue was fixed on one side of the mould and label was placed on the opposite side of the mould. After a skin of wax has formed completely over the surface of the block, its solidification was hastened by careful immersion in cold water, for 15 min. Then the block was removed from the mould. The blocks were prepared for cutting. Finally tissue was cut in the sizes of 3 to 5μm in the form of a ribbon.

The individual sections were gently lowered onto the surface of water at 5^0 C to 10^0 C to remove the folds. The sections were taken on egg albumin coated slides. Slides were kept for drying on a hot plate at 45^0C - 50^0C for 2 hours or more as per requirement[34].

4.16.2: Tissue Collection

After proper dissection of rat; Heart (Myocardium), elastic artery (Aorta) liver and kidney were isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours[33]. The fixed tissues were processed routinely and then embedded in paraffin, sectioned to 3–5 µm thickness, de-paraffinized, and rehydrated using standard techniques. The extent of high fat diet induced variations were evaluated by assessing morphological changes in the myocardium, elastic (Aorta) artery, liver and kidney sections stained with Hematoxylin and Eosin (H and E), using standard techniques.

STATISTICAL ANALYSIS:

- Data was analyzed by using SPSS software version 16.
- All values were expressed as mean ±SD.
- One way ANOVA was used to determine the significance of inter group differences followed by 'Post Hoc t test'.
- Pearson's correlation was done
- $p \leq 0.05$ was considered statistically significant.

REFERENCES:

1) Badyal DK, Desai C. Animal use in pharmacology education and research: The changing scenario. Indian J Pharmacol 2014; 46 (3):257.

2) Charan J, Kantharia ND. How to calculate sample size in animal studies?. Journal Of Pharmacology & Pharmaceuticals 2013 Oct; 4(4):303.

3) Neelima N, Sudhakar M, Lakshmi BV. Antistress activity of ethanolic extract of *Emblica officinalis* fruits in stress induced biochemical and physiological perturbations. Int J Res Pharmacol Pharmacotherap 2014; 3(1):72-79.

4) Patil U.H, Gaikwad D.K. Phytochemical evaluation and bacterial potential of Terminalia Arjuna stem bark. International Journal of Pharmaceutical Sciences and research 2010; 2(3):614-19.

5) Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. International Pharmaceutica Sciencia 2011; 1:103-4.

6) Das KK, Dasgupta S. Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction. Environ Health Perspect 2012; 110 (9):923-26.

7) Mani D.N, Bawnkale D.U, Saroj B.K. Hyperlipidemic model: studying lipid profile in small experimental animal. International Journal of Pharmacy and pharmaceutical Sciences 2012; 4(2):337-40.

8) Pingali U, Fatima N, Murlidhar N. Effects of phyllanthus Emblica extract on endothelial dysfunction and biomarkers of oxidative stress in patients with type 2 diabetes mellitus :a randomized, double-blind, controlled study. Diabetes, metabolic syndrome and obesity: Targets and Therapy 2013; 6:275-84.

9) Munshi RP, Joshi SG, Rane BN. Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance markers for coronary heart disease. Indian journal of Pharmacology 2014 May;46(3):270.

10) http://www.utexas.edu/research/rsc/iacuc/forms/guide04.pdf (accessed on 24.07.2014)

11) htpp://www.myco-instrumentation.com/?equipments=hematology-analyzer-40.

12) Schettler G, Nussel E. Massnahmen Zur prevention der artherosklerose. Arb.Med.Soz.Med.Prav.Med 1975;10: 25.

13) Gordon G.B, Southern A.L. Metabolic effects of alcohol on the endocrine system in "Metabolic aspects of Alcoholisum" C.S. Lieber ed, University park press, Baltimore; pp 249-72.

14) Friedwald WT, Levy RI, Fredricson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin Chem 1972; 18: 499-502.

15) Doumasa BT, Watson WA, Biggs HG. Albumin standards and measurement of serum albumin with bromocerol green. Clin. Chim Acta 1971; 31:87-96.

16) Gomall AG, Bardwill CJ, David MM. Determination of serum proteins by means of Biuret reaction. Journal of Biological Chemistry 1949;177 (2):751-66.

17) Pearlman PC, Lee RT. Detection and measurement of total bilirubin in serum with use of surfactants as solubalizing agents. Clinical Chemistry 1974; 20:447-53.

18) Schumann G,Bonora R, Ceriotti F, Ferard G, Ferrero CA, Franck PFH et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002a; 40: 725-33.

19) Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA, Franck PFH et al.IFCC primary reference procedure for the measurement of catalytic activity concentrations of enzymes at 37°C. Part4. Reference procedures for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med 2002b; 40:718-24.

20) Rosalki SB, Foo AY. Two new methods for separating and quantifying bone and liver alkaline phosphatise iso enzymes in plasma. Clin Chem 1984; 30:1182-6.

21) Gutmann I, Bergmeyer HU. Methods of enzymatic analysis, ed Bergmeyer HU, Acadaemic press NY,1974;4:1794-98.

22) Fabiny DL, Ertingshausen G. Automated reaction rate method for determination of serum creatinine with centrifichem. Clin Chem 1971;17:696-700.

23) Das KK, Nemagouda RS, Patil SG, Saha S. Possible hypoxia signalling induced alteration of glucose homeostasis in rats exposed to chronic intermittent hypoxia-role of antioxidant(Vitamin C) and Ca^{2+}channel blocker(Cilnidipine). Curr. Signal Transduct Ther.2016;11:49-5.

24) Mathews DR, Hoskar JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis Model assessment: insulin resistance and beta- cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 1985; 28(7):412-9.

25) National Committee for clinical laboratory standards, procedures for the collection of diagnostic blood specimens by venipuncture: approved standards.4th Ed. NCCLS documentnH3-A4,Wayne,PA:1998.

26) Sacks BD: carbohydrates in Burtis, C.A. and Ashwood, AR (Eds) Tietz Textbook of Clinical Chemistry. 2nd Ed. Philadelphia. W.B. Saunders Co.1994.

27) Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2):351-58.

28) Marklund & Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. EJB1974; 47:469-47.

29) Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61: 882-88.

30) Sinha A.K. Calorimetric assay of catalase, Anal.biochem.1972; 47:389-94.

31) Roe JH, Koether CA. The determination of ascorbic in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol Chem. 1943; 147: 399-40.

32) Jarger JG, Hattiwale SH, Das S, Dhundasi SA, Das KK. Modified simple method for determination of serum α-tocopherol(Vitamin E). Journal of basic and clinical Physiology and Pharmocology 2012; 23(1):45-8.

33) Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, Battelle Press, Ohio ;1980, pp 194.

CHAPTER 5

RESULTS

Table 5.1: Effect of EEO on Gravimetry in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
Initial body weight(1st day) gms	214.3±7.6	207±5.5	245.3±6.6	221.3±7.2	213±13	3.2	0.058
Final body weight(21st day) gms	235.3±6.6	224.3±6.6	283.6±6.5 [a, b]	241.3±7.09 [c]	239.6±9.5 [c]	9.2	0.002*
% of body weight gain	8.8±0.6	7.6±0.9	13.3±2.8	8.2±0.7	11±0.8	2.1	0.155

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p ≤0.05).

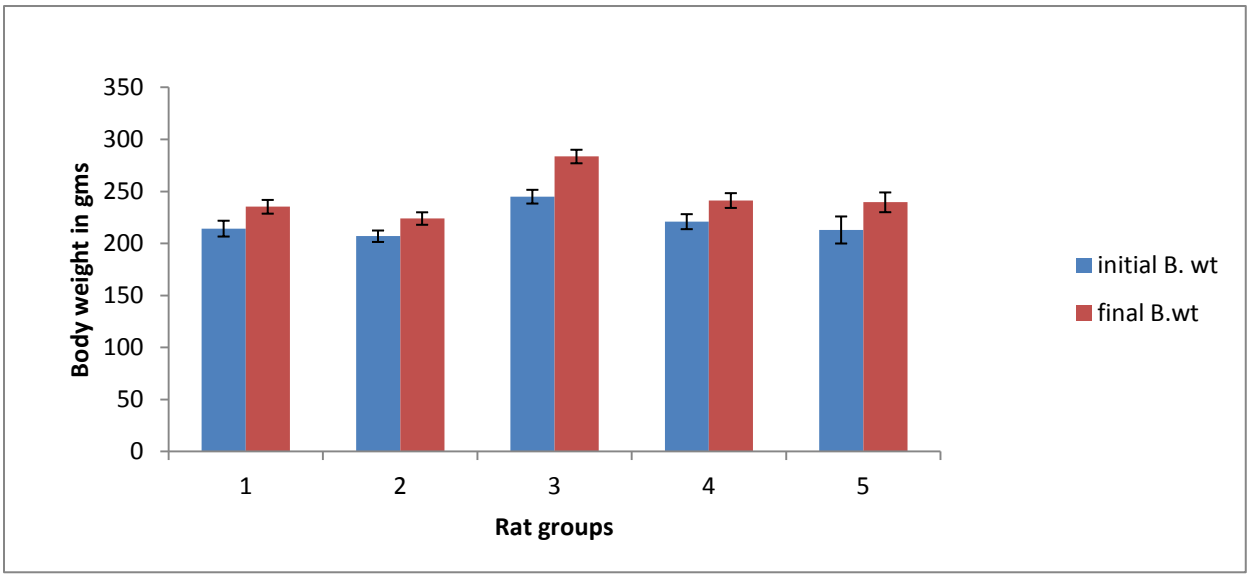


Figure 5.1: Gravimetry: Group 1: control, group 2: is ethanolic extract of *Emblica officinalis* fed (EEO), group 3: high fat fed rats (fat 30%), group 4: high fat diet (fat 30%) + ethanolic extract of *Emblica officinalis* (EEO) fed rats and group 5: high fat diet +aterovastatin. Each value is represented as mean ± SD of two observations in each group.

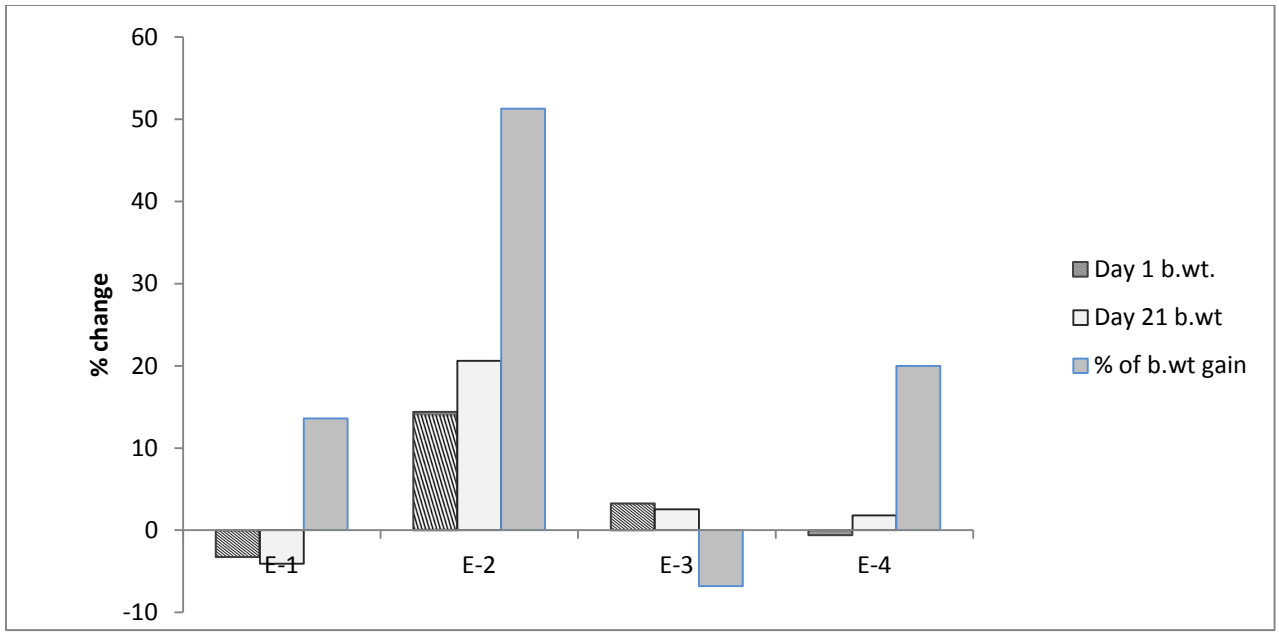


Figure 5.2: % change of initial body weight, final body weight and % change of body weight gain: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5.

Table 5.1 shows significant increase in body weights of rats in group 3 (high fat fed rats, 30% fats) as compared to group 1 (control, 20 % fats) and 2 (EEO supplemented rats) on 21st day. Group 4 (high fat fed rats 30%+EEO) and Group 5 (high fat fed rats30% + atorvastatin) showed significant decrease in final body weight of rats compared to group 3 rats.

However % body weight gain of rats of group 2, 3 and 5 were reduced compared to group 3 even though it was statistically insignificant.

Figure 5.2 depicts percentage difference in initial body weight, final body weight and % change of body weight gain among groups. It shows percentage difference in % of body weight gain between group 1(control) vs group 3(high fat fed rats; 51.3%). After treatment with EEO to high fat fed rats show -6.8% of decrease in % body weight gain compared to control group (E-3; group 1 vs group 4).

Table 5.2: Effect of EEO on organ somatic index in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
Liver gms	8.9 ±0.7	7.8 ±2.1	9.7 ±1.3	7.9 ±0.7	7.8± 0.8	1.2	0.341
Hepatosomatic index	0.04 ± 0.001	0.044± 0.008	0.035 ± 0.002	0.03± 0.004	0.032 ± 0.004	3.5	0.04
Rt Kidney gms	0.8± 0.1	0.6 ± 0.02	0.7± 0.01	0.63 ± 0.05	0.89 ± 0.02	1.9	0.341
Rt renal somatic index	0.003 ± 0.0005	0.003± 0.0001	0.002± 0.0001	0.002 ± 0.0001	0.0033± 0.0005	2.6	0.09
Lt renal gms	0.8± 0.05	0.7 ±0.02	0.76± 0.05	0.76 ± 0.05	0.9 ±0.02	0.88	0.5
Lt renal somatic index	0.003 ±0.0005	0.003 ±0.0001	0.0023± 0.0005	0.0027 ±0.0005	0.003 ±0.0001	0.72	0.5
Heart gms	1 ±0.01	0.8 ±0.02	1.1± 0.2	1.06 ±0.02	0.9 ±0.01	1.21	0.36
Cardiac somatic Index	0.004 ± 0.0005	0.004 ± 0.0001	0.0033± 0.0005	0.004 ± 0.0001	0.033 ± 0.00058	1	0.45

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*$p \leq 0.05$).

We observed no significant differences for hepato, renal and cardiac weight of rats among all groups. There was no significant relation was observed for hepato-somatic index, renal-somatic index and cardiac-somatic index of rats of all groups.

Table 5.3: Effect of EEO on Heart rate variability in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
HR bpm	131± 11	151.6 ± 20	343.6±57[a,b]	258.6 ±44[c]	220.3 ±10[c]	18.56	0.002*
Sympathetic (LF)	0.33±0.05	0.31±0.02	0.59±0.09[a,b]	0.32±0.08[c]	0.31±0.03[c]	10.59	0.001*
Parasympathetic(HF)	0.66±0.05	0.66±0.01	0.39±0.09 [b]	0.67±0.08 [c]	0.64±0.03 [c]	10.54	0.001*
Symp-vagal balance(LF/HF)	0.5±0.01	0.48±0.02	1.5±0.5 [b]	0.50±0.01 [c]	0.54±0.08 [c]	11.2	0.001*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p ≤0.05).

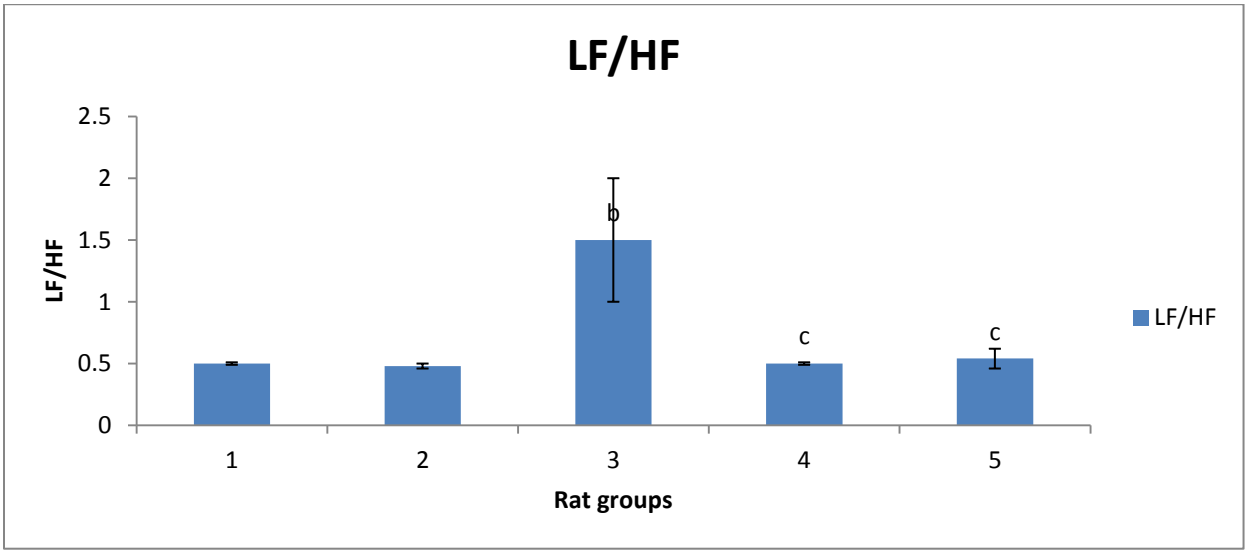


Figure 5.3: Effect of EEO on LF/HF ratio in Rats Fed with High Fat Diet: Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +aterovastatin. Superscript a, b, c, d expresses significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3 and d depicts comparison with group 4.(*p ≤0.05).

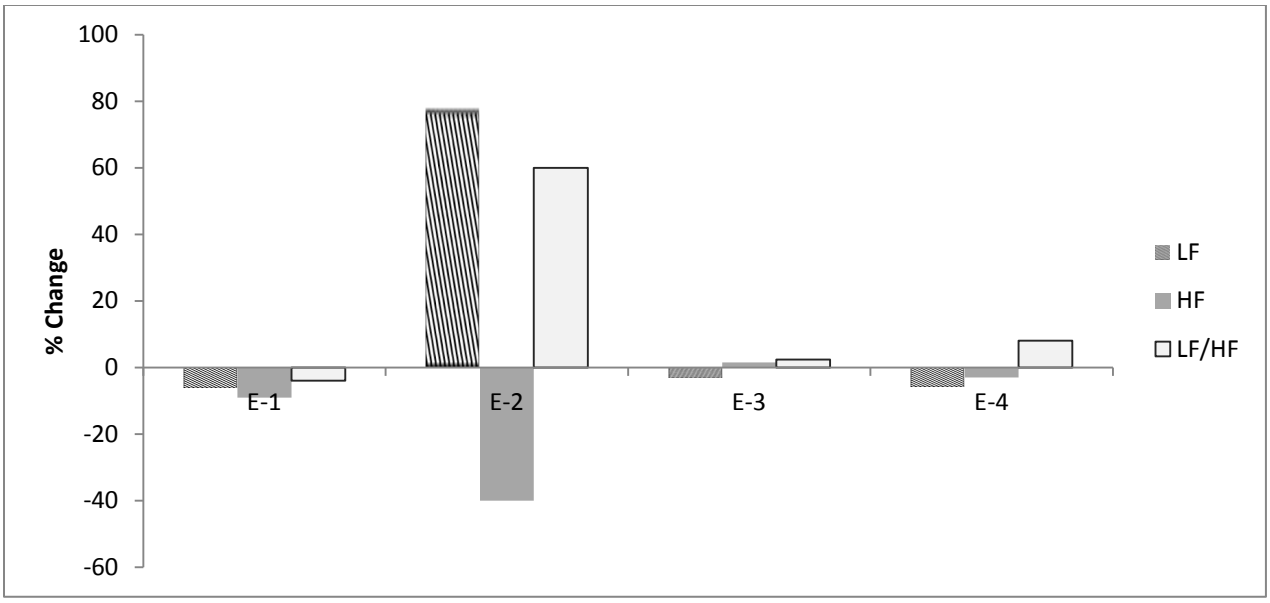


Figure 5.4: Percent change of sympathetic (LF), parasympathetic (HF) and sympatho-vagal balance (LF/HF): E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5. LF: sympathetic HF: parasympathetic, LF/HF: sympatho-vagal balance (LF/HF)

Table 5.3 indicates that heart rate levels were significantly higher in rats fed with high fat diet (30% fat, group 3) compared with control rats (group 1) and rats supplemented with EEO (group 2). Also there were significant low values of HR in rats of group 4 (high fat fed rats 30% +EEO) & group 5 (high fat fed rats 30% + Statin) compared with group 2.

Sympathetic function (LF) was significantly increased in rats fed with high fat diet (group 3) compared to group 1 (control rats) and group 2 (EEO supplemented rats). Group 4 (high fat fed rats 30% +EEO) and 5 (high fat fed rats 30% + atorvastatin) have shown significant lower values of sympathetic function (LF) compared to group 3.

Parasympathetic function (HF) was significantly lesser in group 3 compared to group 2. There were significantly higher values of Parasympathetic function (HF) in group 4 & 5 compared to group 3.

Figure 5.4 depicts percentage difference in sympathetic function (LF), parasympathetic functions and sympatho-vagal balance among groups. Percentage difference for LF between rats fed with

high fat diet (group 3) and control (group 1) was 78.8% (E-2; group 1 vs group 3). After treatment with EEO this difference was -3.03% (E-3; group 1 vs group 4). Percentage difference for HF between rats fed with high fat diet (group 3) and control (group 1) was -40% (E-2; group 1 vs group 3). After treatment with EEO this difference was 1.5%. LF/HF showed 60% difference between control and high fat fed rats groups ((E-2; group 1 vs group 3). Whereas EEO treated high fat fed rats showed significant decrease in percentage difference compared to control rats (E-3; group 1 vs group 4).

Table 5.4: Effect of EEO on Haemogram in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
Hb%	14.8± 0.3	13 ±1.3	10.3 ±1.6[a]	12.4 ±1.1	14.3± 0.6[c]	7.39	0.005*
WBC	9700± 425	5600± 721	6733± 763[a]	6833 ±450	11067 ±1404	3.699	0.042*
Neutro	10± 1.4	14 ±2.25	17.2 ±2.47 [a,b]	8.9±0.8[c]	9.06 ±1 [c]	4.048	0.033*
lympho	85.8 ±1.4	70± 2.25	51.6± 4.27	91 ±2.1	93± 0.045	2.562	0.104
RBC	7.5 ±0.1	7.1 ±0.8	5.8± 1.01	6.9 ±0.9	7.4± 0.04	2.377	0.122
Plt count	8.3± 1.2	8.2 ±0.9[a]	8.6 ±2.7[a]	12.7± 0.9[a,b]	10± 1[b]	16.92	0.000*
PCV	44.5± 1.3	40.9 ±5.6	35.3 ± 4.2	40 ±1.4	40 ±2.5	2.632	0.098
MCV	59± 0.4	57± 3.3	60.7± 3.2	53.6 ±1.5[c]	53.8± 0.6	5.97	0.010*
MCH	19.6 ±0.1	18.2± 0.6	17.6 ±0.4[a]	18.8 ± 1	19.2 ± 0.8	3.962	0.035*
MCHC	33.2 ±0.2	32 ± 1.2	29 ±1.4[a]	32± 1.5	35.7 ±1.9[c]	8.98	0.002*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*$p \leq 0.05$).

Table 5.4 shows that there is significant decrease in HB% and WBC of rats fed with high fat diet (group3) compared to control rats (group1). Rats treated with high fat diet and atorvastatin have

shown significant increase in Hb% compared to group 3 rats. Neutrophil and platelet count were significantly increased in group 3 rats compared to control and group 2 rats. MCV count was significantly decreased in rats supplemented with high fat diet and EEO (group4) compared to rats fed with high fat diet. MCH and MCHC counts were significantly decreased in group 3 rats compared to control rats. No significant differences were observed for lymphocyte count, RBC count and PCV among all groups of rats.

Table 5.5: Effect of EEO on Lipid Profile in Rats Fed with High Fat Diet

Parameter mg/dl	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
TC	121.6±4.7	124.6±6.08	135±3.6^{a,b}	134.3±4.04^{c}	130± 2.5	5.72	0.012*
TG	96± 10	113.6±1.7	124±5.2^{a,b}	114.6±12^{c}	112.3 ±2.08	5.37	0.014*
LDL	68 ±1.3	65.3±4.5	79.8±4.1^{a,b}	75.4±3.4^{c}	71.5± 1.33^{c}	11.78	0.001*
VLDL	19.2±2.02	22.6±0.3	24.8±1.05^{a,b}	22.9±2.4	22.4 ±4.1	5.378	0.014*
HDL	31.3±1.5	36.3±1.5^{a}	30.3±0.5^{b}	35.5±1.5^{c}	36.3 ±2.08c	10.9	0.001*
HDL/LDL	0.45 ±0.02	0.55 ±0.03^{a}	0.38± 0.01^{a,b}	0.47± 0.02^{b,c}	0.51± 0.02^{d}	21.7	0.000*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*$p \leq 0.05$).

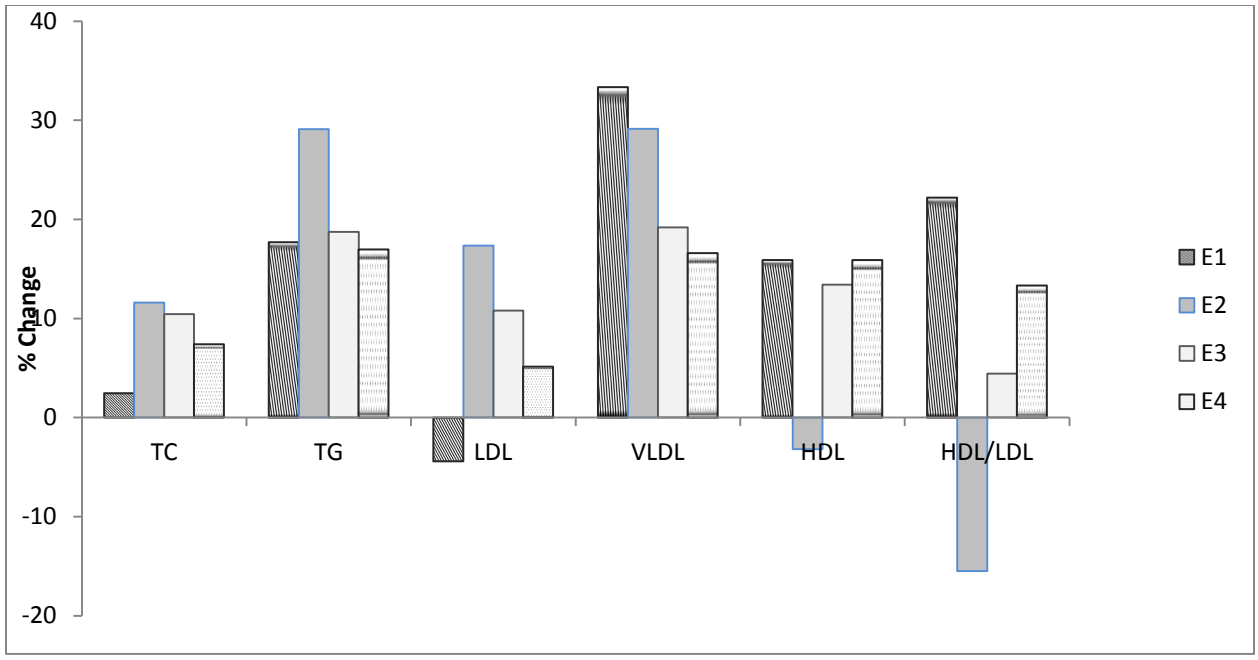


Figure 5.5: Percentage change of lipid profile: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5. TC- total cholesterol, TG- triglycerides, LDL- low density lipoprotein, VLDL- very low density lipoprotein and HDL- high density lipoprotein

Table 5.5 shows TC and TG levels were significantly increased in rats fed with high fat diet compared with control. In case of rats supplemented with high fat diet and EEO showed significant reduction of TC and TG as compared to rats fed with high fat diet. LDL and VLDL levels were significantly higher in rats fed with high fat diet as compared control rats. However group 4 rats showed significant decrease in LDL levels compared to rats fed with high fat diet. HDL levels were found to be significantly lesser in rats fed with high fat diet whereas increase in HDL levels were observed in rats supplemented with high fat diet and EEO.

Figure 5.5 depicts percentage change for lipid profile between groups. Percentage change for TC between control (group 1) and rats fed with high fat diet group (group 3) was 11.6%. After treatment with EEO to high fat diet fed rats, there was significant decrease in percentage difference i.e 10.44% (E-3; group1 vs group4). There was 29.1% increase in percentage change

for TG between control and rats fed with high fat diet (E-2; group 1 vs group 3). After treatment with EEO this percentage difference was significantly decreased to 18.75% between group 1 and group 4(E-3; group1 vs group 4). It was observed significant decrease for HDL in percentage difference between control and high fat fed rats (-3.19%, E-2; group1 vs group3). There was 13.4% significant increase in percentage difference for HDL levels between control rats and high fat fed rats treated with EEO(E-3;groep1 vs group4). Percentage change for HDL/LDL between control (group 1) and rats fed with high fat diet group (group 3) was -15.5%. After treatment with EEO to high fat diet fed rats, there was significant increase in percentage difference i.e 4.4% (E-3; group1 vs group4).

Table 5.6: Effect of EEO on Liver functions in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	**ANOVA**	
						F Value	**P value**
Total protein mg%	0.85± 0.05	0.73 ±0.05	0.73 ±0.05	0.6 ±0.1	0.76 ±0.15	2.5	0.109
Direct mg%	0.53±0.05	0.46±0.05	0.46±0.05	0.4±0.1	0.5±0.1	1.22	0.361
Indirect mg%	0.29±0.01	0.26±0.05	0.26±0.05	0.2±0.0 1	0.26±0.05	1.789	0.208
SGOT U/L	61.6±1.15	60.6±2.5	88.6±3.05 [a,b]	71.6±5.5 [a,b]	71.3±5.8 [a,b,d]	31.149	0.000*
SGPT U/L	73.3±4.6	71±2.6	113.3 ±12 [b]	78.3±2.5 [c]	71±1 [a,c]	22.98	0.000*
S. Protein	5.2±0.1	5.5±0.1	5.4±0.1	5.7±0.1	5.4±0.3	2.304	0.130
S. Albumin	2.8±0.1	3±0.05	2.8±0.1	3±0.2	3±0.1	2.309	0.129
A/G Ratio	0.96±0.02	1.2±0.01	1 ±0.01	1.1±0.02	1.2±0.01	2.125	0.152
S. ALP	154.3±4.5	144.3±6.6 [a]	182.3±4.1 [b]	156±7.2 [a,c]	152±6.08 [a,c]	29.477	0.000*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p $\leq$0.05).

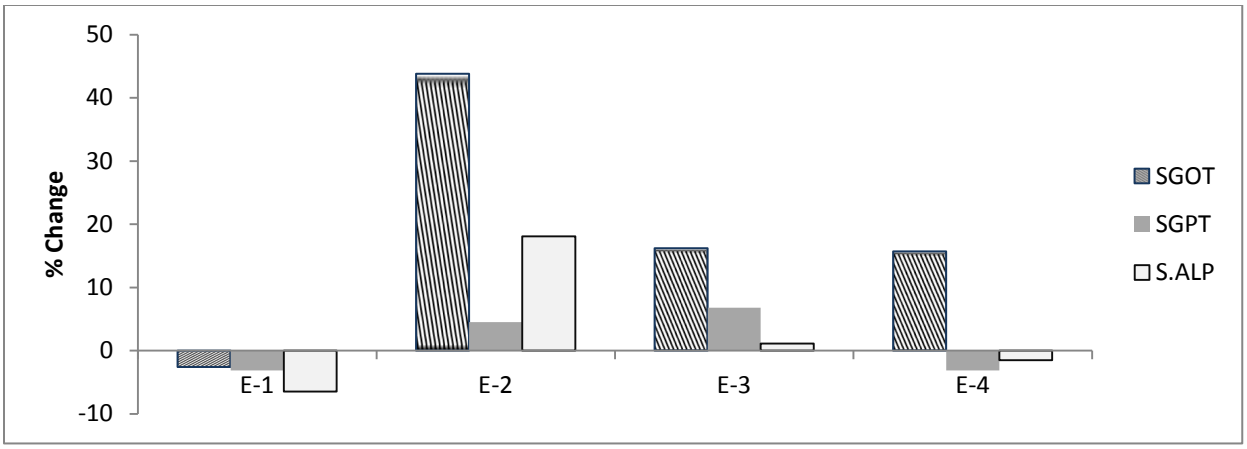


Figure 5.6: Percentage change impact on liver functions: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5. SGOT: serum glutamitc oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase, S. ALP: serum alkaline phosphate.

Table 5.6 shows significant higher levels of SGPT, SGOT and serum ALP in rats fed with high fat diet (group3) compared to control rats (group1). Rats supplemented with high fat diet and EEO(group4) have shown significantly lesser values of SGPT, SGOT and serum ALP compared to rats fed with high fat diet (group3). Also rats treated with high fat diet and atorvastatin have shown significantly lesser values of SGPT, SGOT and serum ALP compared to rats fed with high fat diet (group3). We observed non significant differences for total protein, serum albumin and A/G ratio among all groups.

Figure 5.6 depicts percentage difference of liver functions between groups. Percentage change for SGOT between control (group 1) and rats fed with high fat diet group (group 3) was 43.8% (E-2; group1 vs group3). After treatment with EEO to high fat diet fed rats, there was significant decrease in percentage difference i.e 16.2% (E-3; group1 vs group4). There was 4.5% increase in percentage change for SGPT between control and rats fed with high fat diet (E-2; group 1 vs group 3). After treatment with EEO this percentage difference was significantly decreased to 6.8% between group 1 and group 4(E-3; group1 vs group 4). Percentage change for S.ALP

between control (group 1) and rats fed with high fat diet group (group 3) was significantly higher i.e 18.1% (E-2; group1 vs group3). After treatment with EEO to high fat diet fed rats, there was significant decrease in percentage difference i.e 1.1% (E-3; group1 vs group4).

Table 5.7: Effect of EEO on Kidney functions in Rats Fed with High Fat Diet.

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
Blood Urea mg%	28 ±1	26.3± 1.5	33.6± 2^{a}	27.3± $1.5^{a,c}$	25.8 ±$5.2^{c,d}$	4.004	0.03*
Serum Creatinine mg%	0.8 ±0.1	0.6± 0.05	1.2± $0.1^{a,b}$	0.7± $0.1^{b,c}$	0.9± 0.2^{c}	8.909	0.002*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p ≤0.05).

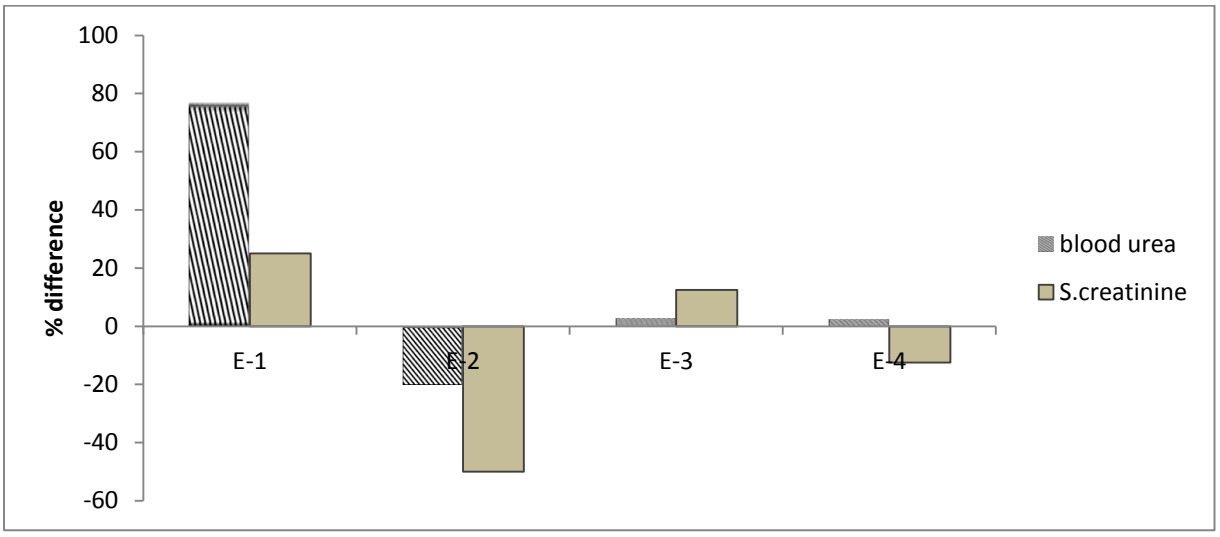


Figure 5.7: Percent change impact on kidney functions: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs grou

Blood urea and serum creatinine levels were significantly higher in rats fed with high fat diet compared (group3) to control rats (group1). Rats treated with high fat diet and EEO (group4) have shown significantly lesser values of blood urea and serum creatinine compared to rats fed

with high fat diet (group3). It was also observed that significant decrease in levels of blood urea and serum creatinine in rats treated with high fat diet and atorvastatin (group 5) compared to rats fed with high fat diet (group3).

Figure 5.7 depicts percentage change impact on Kidney functions. There was decries in percentage change in blood urea and serum Creatinine levels in rats fed with high fat diet supplemented with EEO and statin (E2&E4 respectively)

Table 5.8: Effect of EEO on Oxidative Stress Markers in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
MDA µM/L	0.43±0.08	1.02±0.1	2.3±0.3[a]	1.7±0.2[a,b,c]	2.2±0.4[a,b,c]	70.3	0.000*
SOD U/ml	4.8±0.5	6.4±0.6	3.3±0.9[b]	2.9±0.4[a,b]	3.7±0.8[b]	11.8	0.001*
GSH mg/gm of Hb	13.8±1.04	12.5±1.3	9.4±0.5[a,b]	13.9±1.1[c]	12.9±1.01[c]	8.9	0.002*
Catalase	8.3 ± 0.1	16.1±0.6[a]	4.2±0.05[a,b]	12.3±0.3[a,b,c]	10.8±0.7[a,b,c,d]	261	0.000*
Vitamin C mg/dl	6.2±0.5	7.07±0.4[a]	5.9±0.2	6.7±0.3	5.3±0.4[a,b]	4.4	0.025*
Vitamin E µg/ml	5.8 ± 0.5	6 ±0.4	5 ± 0.9[b]	5.5 ± 0.8	6 ± 0.5	10.8	0.04

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p ≤0.05).

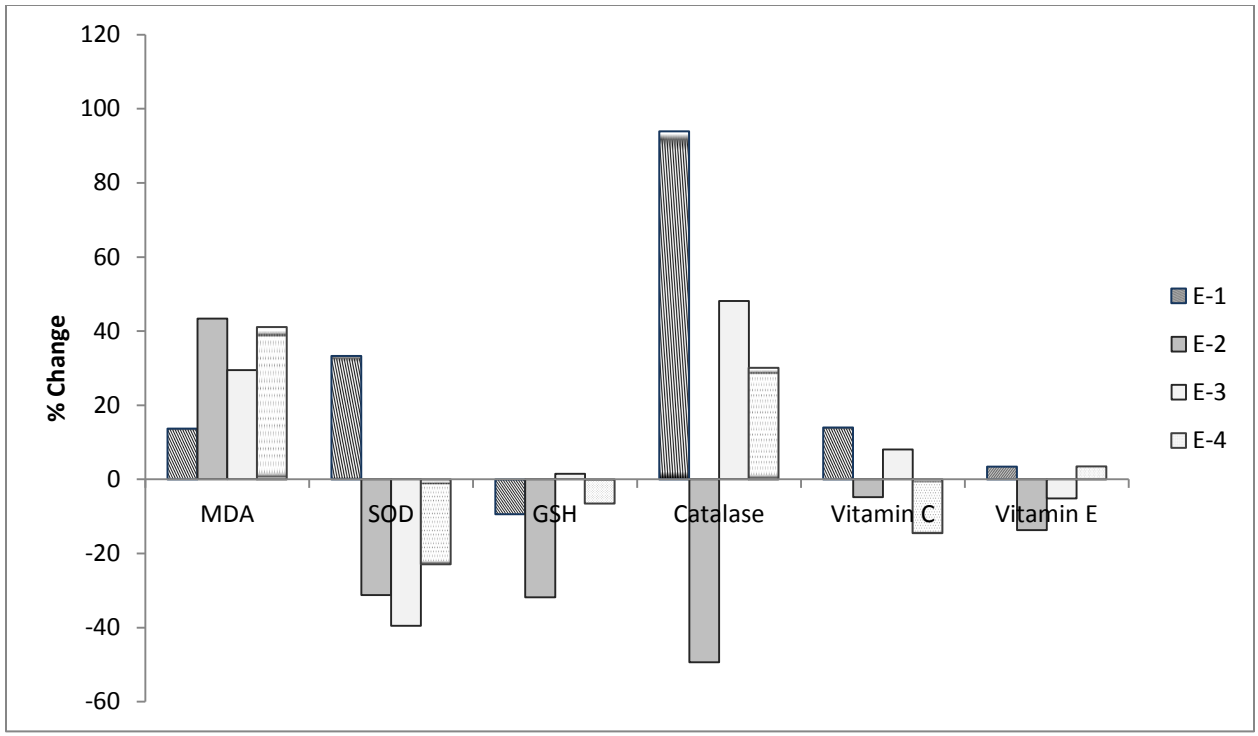


Figure 5.8: Percentage change impact on oxidative stress markers: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5.

Table 5.8 shows Significant decreased levels of SOD, catalase, GSH, vitamin C & E in rats fed with high fat diet indicate to increased lipid Peroxidation and oxidative stress. Supplementation of EEO to hyperlipidemic rats showed significant increase in the activity of superoxide-dismutase, catalase, glutathione and ascorbic acid. We observed significant increase in MDA levels in rats fed with high fat diet (group3) compared to control group (group1). There was significant decrease in MDA levels in rats supplanted with high fat diet and EEO (group4) compared to rats fed with high fat diet (group3).

Figure 5.8 depicts percentage difference of oxidative stress markers between groups. Percentage change for MDA between control (group 1) and rats fed with high fat diet group (group 3) was 43.4% (E-2; group1 vs group3). After treatment with EEO to high fat diet fed rats, there was significant decrease in percentage difference i.e 29.5% (E-3; group1 vs group4). There was -31.2% increase in percentage change for SOD between control and rats fed with high fat diet (E-2; group 1 vs group 3). After treatment with EEO this percentage difference was significantly

decreased to -39.5% between group 1 and group 4(E-3; group1 vs group 4). Percentage change for GSH between control (group 1) and rats fed with high fat diet (group 3) was -31.8% (E-2; group1 vs group3). After treatment with EEO to high fat diet fed rats, there was significant increase in percentage difference i.e 1.5% (E-3; group1 vs group4). Catalase levels have shown significant decrease in percentage difference i.e -31.8% between control group and rats fed with high fat diet (E-2; group1 vs group3). After treatment with EEO to high fat fed rats (group4), the percentage difference was increased to 48.1% (E-3; group1 vs group4). Percentage change for vitamin C between control (group 1) and rats fed with high fat diet (group 3) was -4.83% (E-2; group1 vs group3). After treatment with EEO to high fat diet fed rats, there was significant increase in percentage difference upto8.06% (E-3; group1 vs group4). There was -13.7% decrease in percentage change for vitamin E between control and rats fed with high fat diet (E-2; group 1 vs group 3). After treatment with EEO this percentage difference was significantly increased to -5.17% between group 1 and group 4(E-3; group1 vs group 4).

Table 5.9: Effect of EEO on Nitrosative stress markers in Rats Fed with High Fat Diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
NO µmol/L	4.5±0.6	8.2±0.7[a]	2.5±0.6[a,b]	5.2±0.48[b,c]	5.6±0.5[b,c]	31.94	0.000*
NOS3 ng/ml	30 ±2.7	29.8 ± 1.4	30.9± 2.3	31.1±0.4	29.8±1.7	0.297	0.873

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*p ≤0.05).

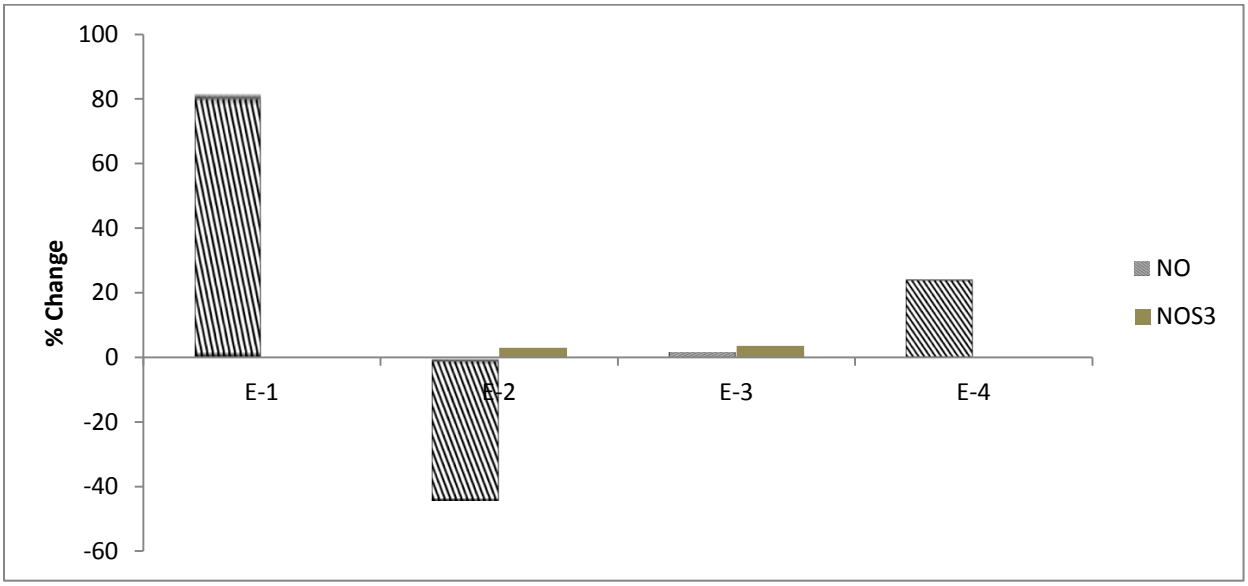


Figure 5.9: Percentage change impact on Nitrosative stress markers: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5. NO: nitric oxide, NOS3: nitric oxide synthase 3.

Table 5.9 shows unaltered NOS3 protein with decreased NO in high fat fed rats indicate a possible alteration of vascular pathophysiology probably through oxygen sensing cell signaling pathway. Supplementation of EEO was found to be beneficial to regulate NO production.

Figure 5.9 depicts percentage difference of Nitrosative stress markers between groups. Percentage difference for NO was -44.4% between control group and rats fed with high fat diet (E-2; group1 vs group3). After treatment with EEO to rats fed with high fat diet, percentage difference was increased to 1.5% (E-3; group1 vs group 4). There was 3.7% change for NOS3 between control and rats fed with high fat diet (E-2; group 1 vs group 3). After treatment with EEO this percentage difference was increased to 3.6% between group 1 and group 4(E-3; group1 vs group 4).

TABLE 5.10: Effect of EEO on glucose homeostasis in rats fed with high fat diet

Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	ANOVA	
						F Value	P value
FBS mg/dl	96.3 ±0.5	100± 2	129 ±1.5[a]	115.3 ± 1.15[b,c]	116 ±2[c]	27.26	0.000*
PPBS mg/dl	86.3± 0.5	96.3± 4.7	115.3 ±1.15[a]	102± 7.2[b,c]	98 ±2[a,c]	20.74	0.000*
Insulin µU/ml	5.8 ±0.5	6.4 ±0.6	5.2± 0.5[b]	5.6± 0.3	5.6 ±0.4[c]	2.33	0.01*
HOMA Index	1.5 ±0.05	1.48± 0.05	1.7 ±0.2[a,b]	1.6± 0.05	1.59 ±0.05[c]	1.28	0.03*

Values are expressed as mean ±SD. ANOVA followed by Post Hoc 't' test. Group 1: control, group 2: supplemented with ethanolic extract of *Emblica officinalis* , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of *Emblica officinalis* fed rats and group 5: high fat fed rats +atorovastatin. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group 1, b depicts comparison with group 2, c depicts comparison with group 3and d depicts comparison with group 4.(*$p \leq 0.05$).

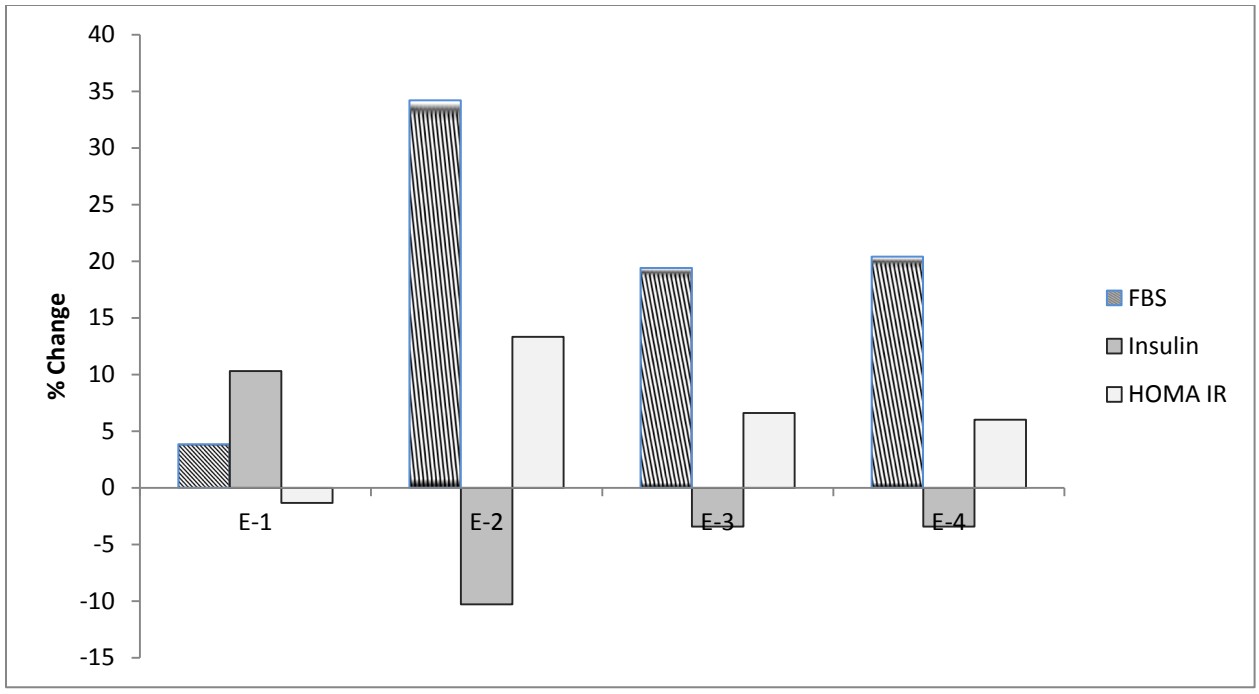


Figure 5.10: Percentage change impact on FBS, Insulin and HOMA IR index: E-1: group1 vs group 2, E-2: group 1vs group 3, E-3: group 1 vs group 4 and E-4: group 1vs group 5. FBS: fasting blood sugar.

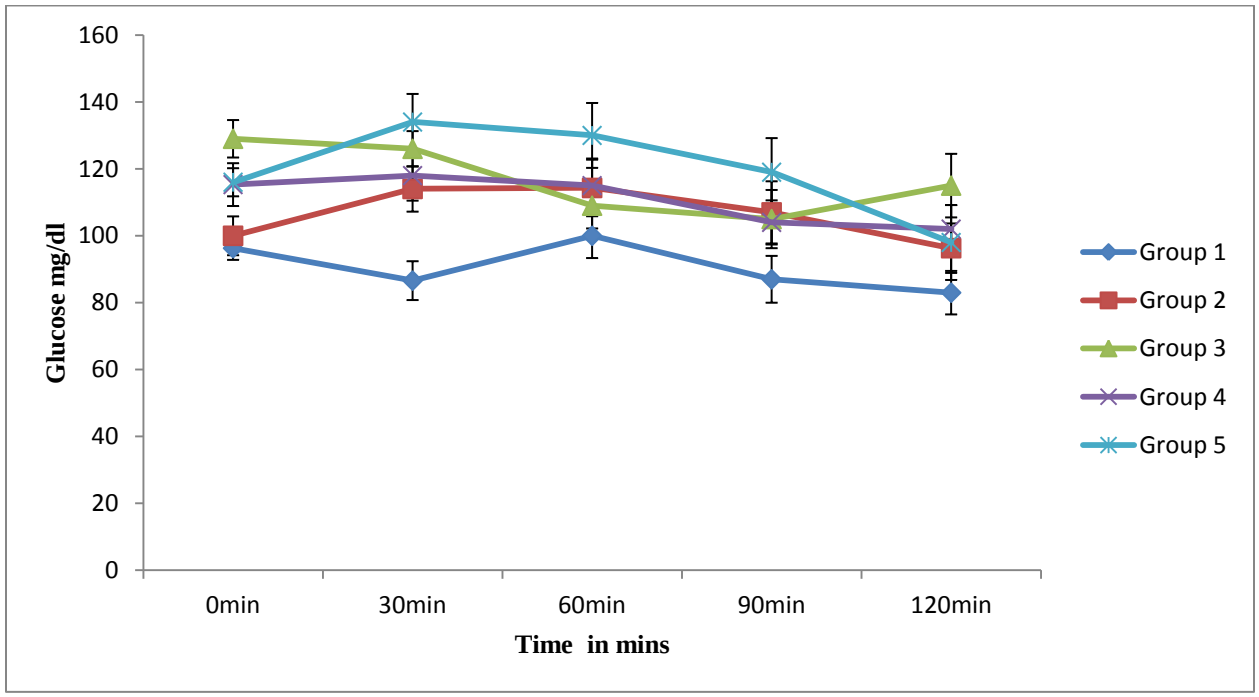


Figure 5.11: Oral glucose tolerance test among all groups: Group 1: control, group 2: supplemented with ethanolic extract of Emblica officinalis , group 3: high fat fed rats, group 4: high fat diet + ethanolic extract of Emblica officinalis fed rats and group 5: high fat fed rats +atorovastatin

Sub chronic evaluation on glucose homeostasis

Table 5.10 shows that FBS and PPBS levels were significantly increased in rats fed with high fat diet as compared to control and rats fed with EEO (group 2), whereas rats treated with high fat diet and EEO have shown significant decrease of FBS and PPBS as compared to rats fed with high fat diet. Fasting insulin levels were found to be significantly lesser in rats fed with high fat diet as compared to control and rats fed with EEO (group 2). Decreased insulin levels are concomitant of increased HOMA-IR index in rats fed with high fat diet compared to control and rats fed with EEO (group 2) whereas an increase in insulin levels with concomitant decrease in HOMA IR index were also observed in a rats fed with high fat diet & EEO as compared to rats fed with high fat diet.

Figure 5.10 depicts percentage difference of FBS, insulin and HOMA IR between groups. Percentage difference for FBS was increased to 34.2% between control group and rats fed with high fat diet (E-2; group1 vs group3). After treatment with EEO to rats fed with high fat diet, percentage difference was decreased to 19.4% (E-3; group1 vs group 4). HOMA IR levels have shown increase in percentage difference up to 13.33% between control group and rats fed with high fat diet (E-2; group1 vs group3). After treatment with EEO to high fat fed rats have shown decrease in percentage difference up to 6.6% (E-3; group1 vs group 4).

Oral glucose tolerance test among all groups: Figure5.11

Control rats showed fasting glucose within normal limits and highest peak value reached with in 1 hour. Then blood glucose level reached back to normal by 2 hours. It shows normal glucose tolerance. Rats fed with high fat diet have shown higher blood glucose at initial point and reached to peak within 1 hour but didn't reached to initial blood glucose level till 2 hours which clearly indicates impaired glucose tolerance. Rats supplemented with high fat diet and EEO has shown improved glucose tolerance.

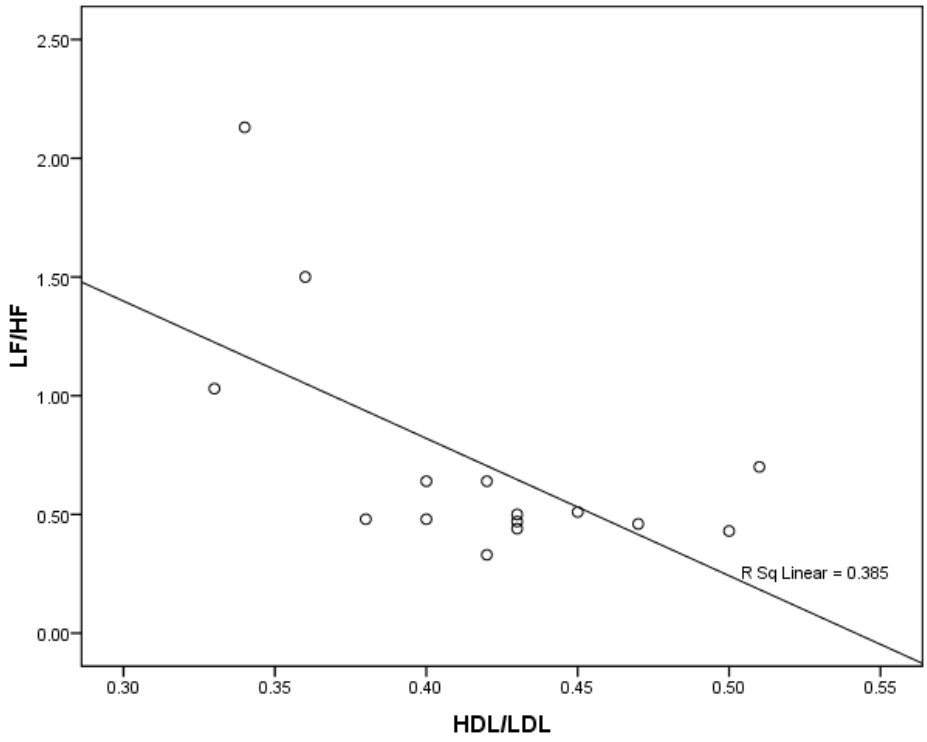


Figure 5.12: Correlation between sympatho-vagal balance (LF/HF ratio) and HDL/LDL ratio in all groups of rats (r= -0.620, p<0.05*)

The respective correlations between sympatho-vagal balance (LF/HF ratio) and HDL/ LDL ratio among all groups of rats have been given in Fig 5.12

Fig 5.12 depicted significant negative relationship between sympatho-vagal balance (LF/HF ratio) and HDL/ LDL ratio (r= -0.620, p<0.05*) among all groups of rats.

HISTOPATHOLOGY

5.13: HISTOPATHOLOGY

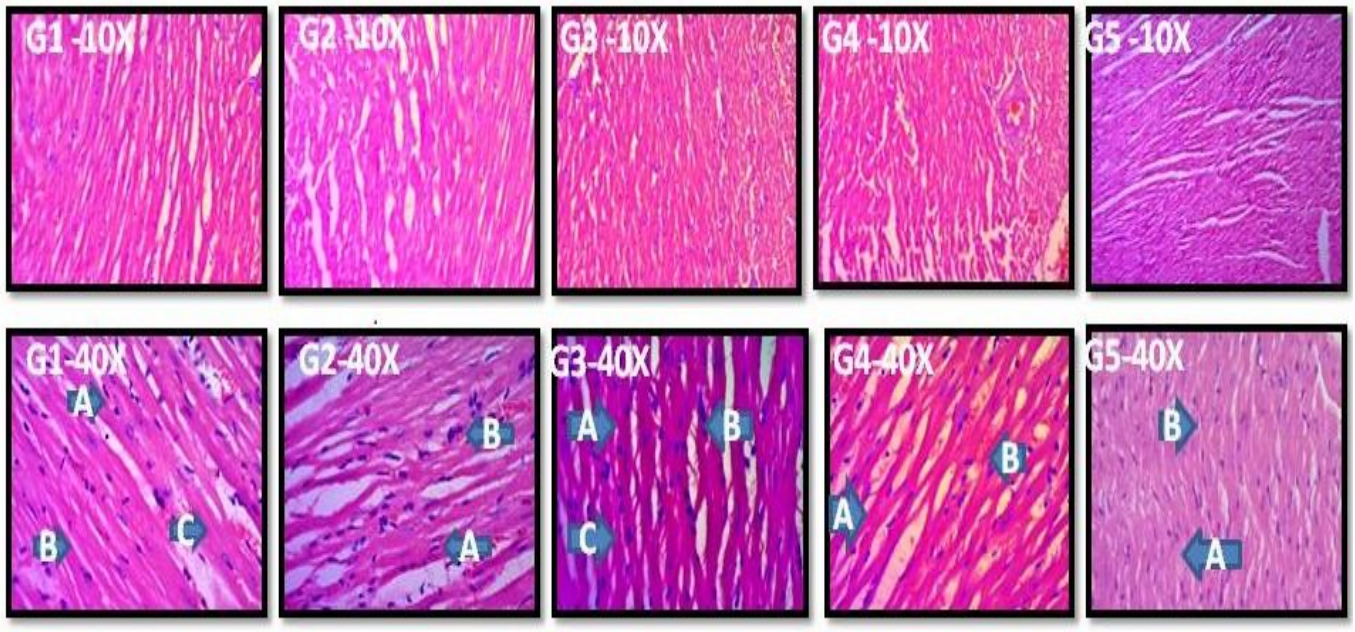


Figure 5.13.1: Histopathology of Myocardium: Microscopic structure of myocardium in all groups are stained with H & E (10 x & 40x) depicted in two rows. Control group (G1): **A** central placed nucleus, **B** intercalated disk, branching and anastomosing striated muscle and **C** capillary. G2 is rats fed with EEO. **A** Normal myocardium , **B** Prominent and central placed nucleus. G3 is rats fed with high fat diet **A**: myocardial hypertrophy with multiple nucleus, **B** degeneration & **C** capillary congestion. G4 is rats fed with high fat diet with EEO **A** Normal myocardium. **B** Mild capillary congestion. G5 is rats fed with high fat diet with Statin. **A** Normal myocardium and **B.** Prominent and central placed nucleus.

Histopathological architecture of myocardium showed prominent intercalated discs and centrally placed nucleus in control group. There was a mild focal myocardial hypertrophy and degeneration and capillary congestion in group 3. And there was no evidence of focal myocardial hypertrophy, degeneration and capillary congestion in treated group's i.e. 2, 4 & 5.

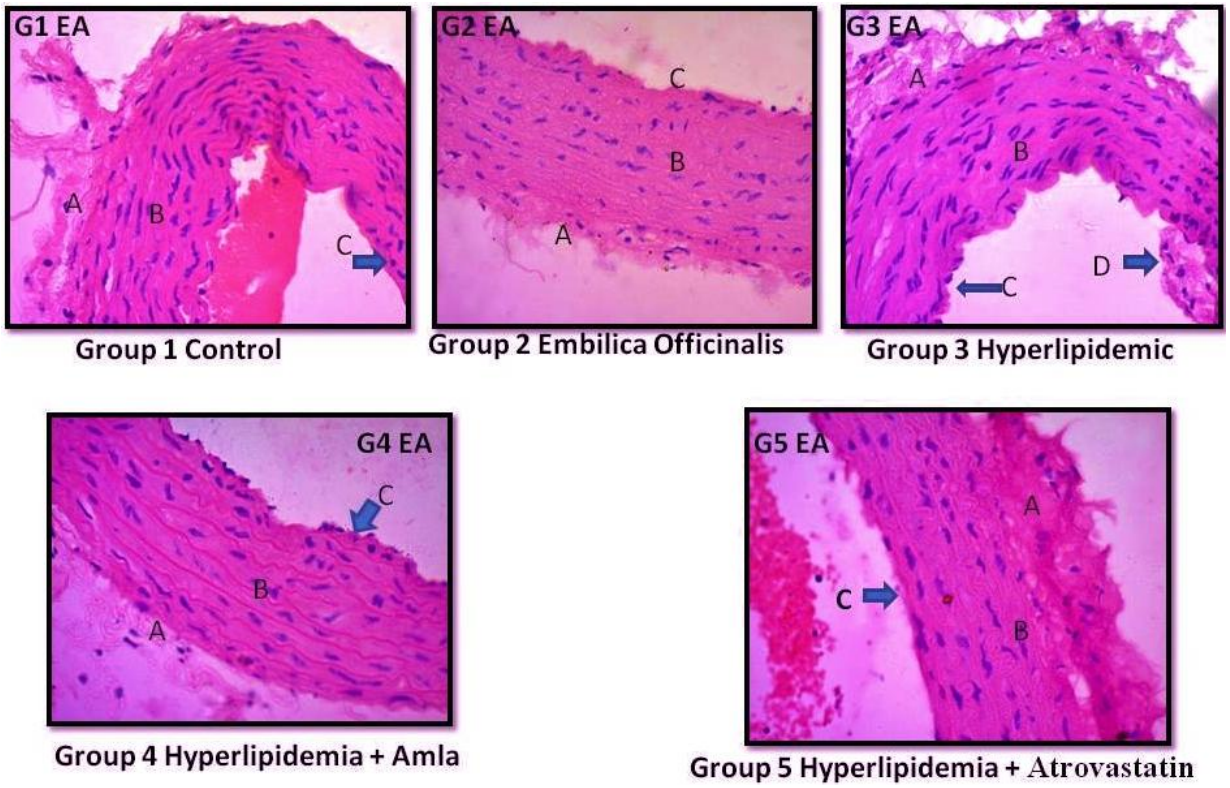


Figure 5.13.2: Histopathology of Elastic Artery: Microscopic structure of elastic artery in all groups are (stained with H & E, in 40x) depicted. Control group (G1) A. Tunica Adventitia, **B.** Tunica media showing spindle shape nucleus with smooth muscle fiber and **C.** Tunica intima with normal endothelium. G2 is rats fed with EEO: showing normal architecture of **A** Tunica adventitia, **B** Tunica media. C Tunica intima G3 is rats fed with high fat diet **A:** Tunica Adventitia, **B**: Morphological alterations of smooth muscle cells with its nuclei in Tunica media**, C:** Tunica intima and **D** tunica intima was lined by endothelium with early changes of atherosclerosis. G4 is rats fed with high fat diet with EEO: **A** Tunica adventitia, **B** Tunica media, & C Tunica intima. G5 is rats fed with high fat diet with Statin **A** Tunica adventitia, **B** Tunica media and **C** Tunica intima. G4 & G5Treated groups all the three layers of artery showing normal architecture

Microscopic architecture of elastic artery of all the three layers ie tunica intima, media and adventitia showed normal in control group (Group 1). Rats fed with high fat diet (group 3) were presented with morphological alterations in the nuclei of smooth muscle cell of tunica media. The tunica media showed degeneration in round shape and hyperplasia of the smooth muscle cell with round nuclei, also elastic fibers showing moderate thickening. The microscopic architecture of tunica intima lined by endothelial cells showing early changes of atherosclerosis in group 3, whereas Group 2, 4 and 5 (treated groups) tunica media and tunica intima microscopic architecture normal but mild thickening in the layer of tunica media

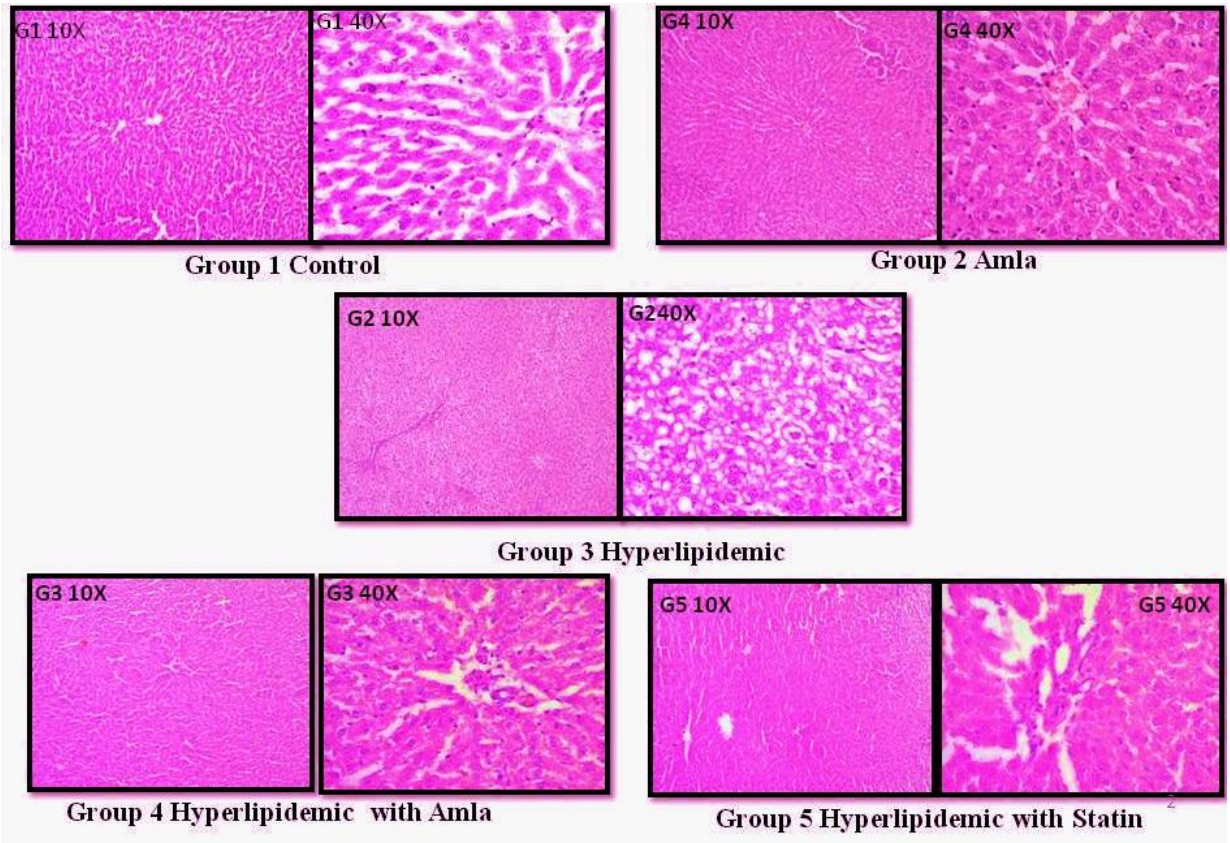


Figure 5.13.3: Histopathology of Liver: Microscopic architecture of Liver stained with H & E in 10X and 40X of all the groups rats. G1 control group 10X & 40X section of liver shown normal hepatic architecture of hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation whereas G2, G4 & G5 groups has shown prominent sinusoidal dilatation. G3 group architecture of the liver with enlarged hepatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion

Histopathology of liver in control group 1 rats has shown normal hepatic architecture compressed of hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation. Group 3 rats have shown lobular architecture of the liver with enlarged hepatocytes containing microvescicular and macrovesicular fatty changes with sinusoidal congestion. Whereas group 2, 4 and 5 has shown prominent sinusoidal dilatation

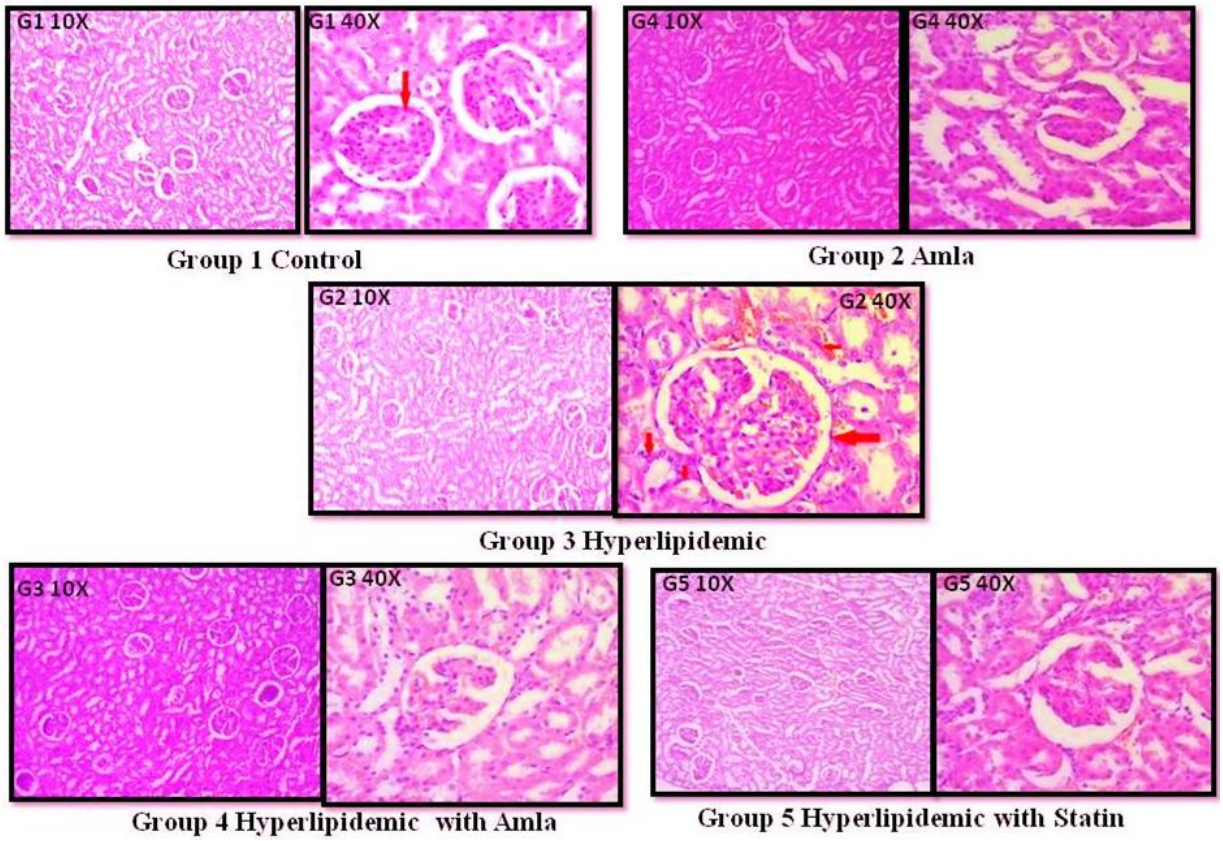


Figure 5.13.4: Histopathology of Kidney: Microscopic architecture of Kidney in all the groups are (stained with H&E in 10X and 40X) depicted. G1 Control Group showing Normal Parenchyma of Kidney and No Histopathologic Changes (H and E stain 10X & 40X) Glomerular , Space of Urine, Bowmen's Capsule, Proximal tubule, Distal tubule, Efferent arteriole, Afferent Arteriole. G2 Parenchyma of kidney showing normal architecture. G3 group architecture showing Focal Glomerular Lesions Including Thickening of the Glomerulus and Normal Renal (DCT) Tubules whereas, G4 & G5 groups has shown mild thickening of glomerulus with normal architecture of kidney

Histopathology of renal corpuscles exhibited normal appearance in Groups 1 and 2 whereas Group 3 showed prominent increase in the glomerular capillaries and also showed focal glomerular lesions including thickening of glomerulus and completely decreased space between glomerulus and Bowmen's capsule. Group 4 and 5 showing mild alterations of architecture of kidney with normal renal corpuscles

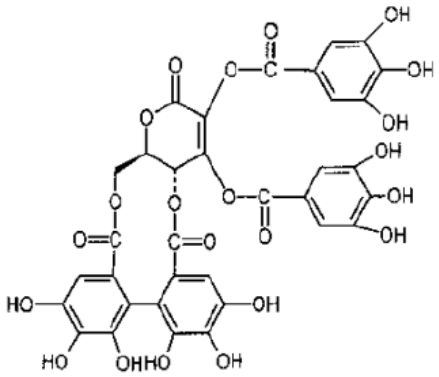
CHAPTER 6

DISCUSSION

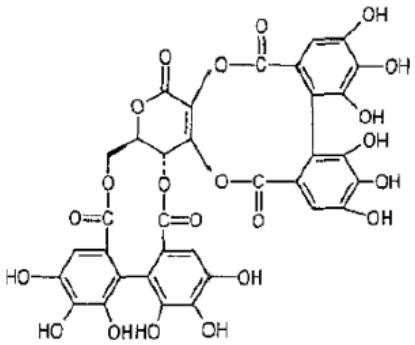
6.1: Phytochemistry:

Extraction of plant or fruits process gives an idea about amount of extract present in a definite quantity of drug. The extractability also provides tool for the quality control of plant drug. The acute and chronic toxicity of drug extraction provides basic information about its useful properties and lethal dose (LD 50). Dose of drug and route of administration can be decided by observing acute and chronic toxicity. It is well known fact that many herbal drugs have various medicinal properties due to active principles like alkaloids, glycosides, reducing sugars, tannins and flavonoids. Bioactive compounds can be extracted with many solvents like alcohol, water, petroleum ether and benzene[1].

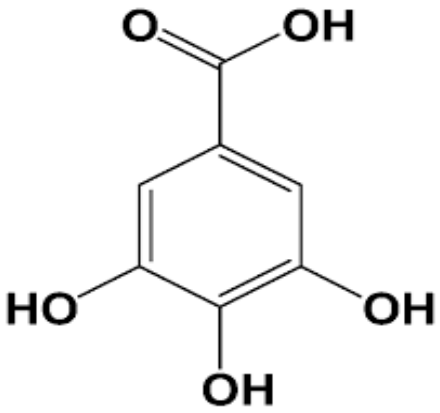
Phytochemical analysis in our study has shown that *Emblica officinalis* (Amla) does not have any toxic ingredients. Amla or Indian gooseberry has been playing an important role from ancient times in traditional medicine, Ayurveda and in tribal medicine. The major group of phytochemicals such as tannins, tannoids, flavonoids and other polyphenolic compounds extracted from Amla has been screened for diverse biological and biopharmaceutical investigations from last few decades[2]. Some important Amla phytochemicals like gallic acid, ellagic acid, emblicanin A, emblacani B, quercetin, phyllantine, phyllantidine have been established of possessing many biological activities like antioxidant, hypolipidemic, hepatoprotective, cardioprotective, antimicrobial, anti-inflammatory, antidiabetic, radioprotective, wound healing activities[3].



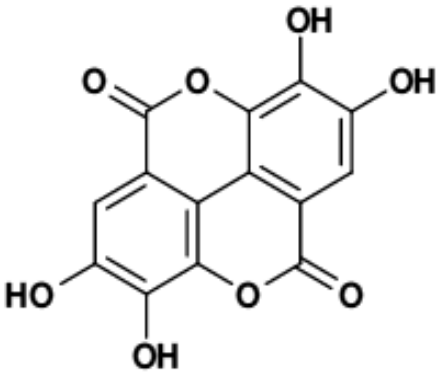
Emblicanin A



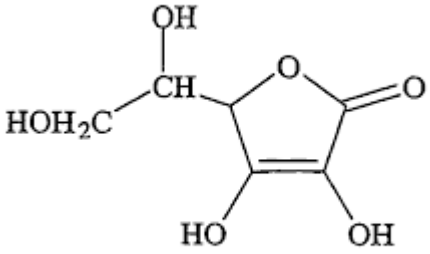
Emblicanin B



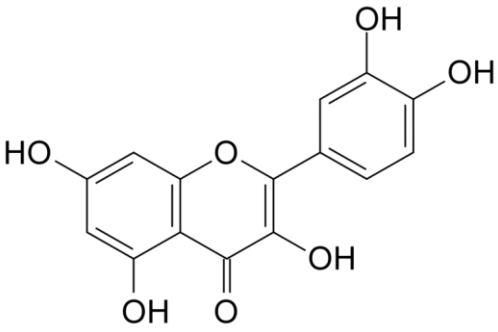
Gallic acid



Ellagic Acid



Ascorbic Acid



Flavonoids

Figure 6.1: Active compounds of *Emblica Officinalis*

6.2: Gravimetry:

Hyperlipidemic diet showed an increase in total body weight and % of body weight gain of rats fed with high fat diet. A possible explanation for the influence of high fat diet on weight gain is that diet induces a positive fat balance due to the loss of adjustment between fat oxidation and consumption. In the long term, this positive accumulation can lead to weight gain and hence longer the duration of the diet, greater is the gain of body weight[4]. Significant reduction of body weight in rats with supplementation of EEO indicated a possible interference of protein metabolism[5].

6.3: Autonomic functions:

HRV is a mainly used as a measure for the imbalance between sympathetic and parasympathetic autonomic activity. Its decrease is a sign of sympathetic activity dominance. Decrease in HRV and sympatho-vagal imbalance indicate presence of cardiovascular disease with incidence of ischemic heart diseases[6].Obesity and autonomic nervous system are interrelated such as, 10% increase in body weight is linked with decrease in parasympathetic tone and rise in heart rate[7]. Chin- Hua Fu et al, reported that baroreceptor sensitivity is negatively correlated with LDL cholesterol. The impaired endothelium-dependent arterial dilatation in vessel walls caused by higher lipid levels may also change the baroreflex capacity[8]. The sympatho vagal imbalance is considered as main pathophysiological basis of metabolic disorder in diabetes mellitus[6,9]. In the general population HRV shows negative correlations with HDL-C levels[6]. This finding is in accordance with our result.

Chaar LJ et al, hypothesized that intake of a high-fat diet may cause changes in neuropeptide levels within autonomic nuclei in the hypothalamus and brainstem which leads to the development of obesity-associated sympathetic hyperactivity and autonomic imbalance[10].

Roy A et al showed that hypercholesterolemia is associated with decreased 24 hour heart rate variability. Decreased heart rate variability is a valuable predictor of CHD[11]. HR is an indicator of sympathetic nervous system. Sympathetic nervous system activity increases with fat induced calorie intake[12]. It has been reported that rats on hyperlipidemic and hypercholesterolemia diet showed significant increase in HR as compared to the control group[12]. Decreased HF power and increase LF power in our study indicates LF/HF imbalance which shows cardiovascular abnormality due to autonomic malfunction in high fat fed rats.

It was reported that combined hyperlipidemia was related with decreased HRV which was partially reversible with statins and fibrates[13]. Our findings are in agreement with the findings of earlier studies where long term atorvastatin therapy in hypercholesterolemic subjects led to increase in HRV[13, 14]. C. D. S. Tremarin et al reported an increase in heart rate and LF:HF ratio in high lipid diet supplemented rats, suggesting an increase in the sympathetic drive[15]. But our results are contrary to these findings. Farah Khaliq mentioned that after using some plant based compounds, there was significant improvement in autonomic functions as well as cardiac health in diabetic rats[16]. In our study Amla supplemented high fat diet treated rats showed improvement of HRV which suggest its beneficial role to regulate autonomic function. Sub chronic supplementation of some indigenous plant extract to alloxan induced rats have shown significant improvement in sympatho-vagal balance by increase of heart rate variability and regaining baroreflex sensitivity[17]. Our results support above mentioned findings.

6.4: Haematology:

High fat diet can lead to overweight and obesity which subsequently enhance low grade chronic inflammation along with increased levels of TNF-α, IL-1 and IL-6. Bone marrow

mesenchymal stem cells can be affected by these cytokines which results in induction of NF-kB and inhibition of PPAR-γ. It further causes suppression of bone marrow adipogenesis[18].

High fat diet induced rat model have produced significant altered hematopoietic and lymphpoietic functions in the bone marrow and thymus[19].

Benjamin J et al reported in their study that high fat diet suppresses B-lympopoiesis by declining lymphocyte count[20], similar results were observed in our study although lymphocyte count was not statistically significant in high fat fed rats.

Our observation to hematological profile of all group rats indicate that ethanolic extract of *Emblica Officinalis* has beneficial effects on Hb%, Neutrophils, platelet count. Remaining unchanged hematological profile (MCV, MCHC) shows that EEO has no adverse effect on haematopoiesis which supports study conducted by Arumugam et al[21].

6.5: Lipid profile:

In our study high fat diet rats have shown significant alterations in lipid profile. High fat diet increased triglycerides level and leads to hardening of arteries[22]. High fat diet may cause excess hepatic triglycerides due to increased synthesis of triglycerides and increased de nova lipogenesis[23]. Dysfunctional adipose tissue is insensitive to insulin and has decreased hormone sensitive lipase activity compared with normal functioning adipose tissue. Because of this, there is increased breakdown of intracellular TG and increased levels of free fatty acid into the circulation causing fatty infiltration in the liver, muscles and pancreatic beta cells[24]. Subsequently increase in hepatic FFA levels contributes to increased hepatic triglycerides synthesis as well as elevated concentrations of very low density lipoproteins (VLDL) particles. Cholesterol ester transfer protein (CETP) exchanges triglycerides from VLDL to cholesterol present in HDL and LDL, resulting to form cholesterol rich atherogenic VLDL particles. These

HDL particles undergo many modifications and get cleared off by kidneys causing decreased levels of HDL[25].

Various studies reported that rather than cholesterol concentration in different lipoproteins, the size and composition are found to be important in atherogenesis. Lipid ratios have also been found to indicate an atherogenic risk and are said to better predictors of coronary artery diseases than lipids alone. Among the lipoprotein subclasses, disproportionate amounts of small, dense LDL particles and small HDL particles cause atherogenic profile due to a high susceptibility to oxidation[26].

Emblica officinalis supplemented to HFD fed rats in our study showed significant changes in lipid profile by redistribution of lipoproteins possibly through its bioactive compounds like flavonoids which are capable to prevent LDL oxidation[2]. It has also been reported that ethanolic extract of *Emblica officinalis* may reduces cholesterol synthesis by inhibiting HMG CO-A reductase activity[27]. Another hypothesis can be framed as the possible polyphenolic compounds of *Emblica officinalis* might have interfered and counteracted lipid peroxidation[27].

Also standardized extract of amla fruit increases cardiac glycogen levels and decreases LDL levels. The mechanism may include increase in LCAT (Lecithin Cholesterol acyltrans): the enzyme responsible for ester transfer to HDL[28]. Similarly there may be increase in lipoprotein lipase activity. This increased enzymatic activity increases the ability of muscle fibers to oxidize fatty acids coming from VLDL, TG. This process of cholesterol removal from circulation is termed as reverse cholesterol transport[28]. It was reported that Polyphenols present in amla fruit juice (541.3 mg gallic acid equivalent / 1 gm extraction) might be responsible for lipid lowering effects of fruit juice which may explore cardio protective effects[29].

It was reported that atherogenic ratio was significantly increased in high fat diet induced rats compared to control rats. There was significant decrease in atherogenic ratio in hyperlipidemic rats treated with polyherbal formulation (OB- 6). [Polyherbal formulation (OB-6) is extracts of six medicinal plants *Cassia angustifolia, Nigella sativa, Phyllanthus amarus*, *Emblica officinalis, Zingiber officinale* and *Terminalia chebula*] which corroborates with our findings[22].

6.6: Liver functions:

Adipose tissue being an active endocrine organ secretes proinflammatory markers i.e adipokines. Visceral adipose tissues are more resistant to leptin hence contains higher triglycerides than peripheral fat stores[30]. The lipolysis of triglycerides in visceral adipose tissue releases free fatty acids into the portal vein from where they are sent to liver[31].

Insulin acts to decrease glucose production by two ways i.e direct action upon the liver or indirectly through free fatty acids. Hyperinsulinemia, normally represses lipolysis and the release of free fatty acids; however, visceral adiposity has shown resistance to the antilipolytic effects of insulin. Therefore, when excess free fatty acids reach the liver via the portal circulation, they interfere with the mechanism by which insulin inhibits hepatic glucose output[32]. An excess of free fatty acids from obesity and increased visceral fat makes negative impact on insulin-glucose balance within the body[30,31,32].

Nutrition overload occurred due to high fat diet cause an increased efflux of non-esterified fatty acids (NEFAs) and release of cytokines and adipokines. Further this may result in ectopic deposition of fat in the liver. Fat accumulation in liver may lead to toxic effect[33].

Nonalcoholic fatty liver disease (NAFLD) includes many liver anomalies related to the accumulation of fat in hepatocytes, like simple steatosis which is a benign condition that may progress to serious liver cirrhosis[34,35] . Nowadays two pathophysiological model for NAFLD are considered i.e "two hits" model. First, is accumulation of triacylglycerides (TAG) and free fatty acids (FFA) in the liver as a result of changes in the influx, synthesis, oxidation and transport of fatty acids. The second hit, triggered by the first hit, involves oxidative imbalance, decreased hepatic ATP production, insulin resistance and induction of proinflammatory, lipid peroxidation and activation of inflammatory pathways as NF-kB and JNK[35,36] .

Liver function tests in our study have shown unaltered levels of few parameters like total protein, serum albumin and A/G ratio in rats fed with high fat diet. And after treatment with EEO to high fat fed rats have shown no alterations in these liver functions which indicates *Emblica Officinalis* (Amla) is non toxic to Liver function tests and may be used as safe ingredients. Serum ALP, SGOT & SGPT are definitive indicators of liver parenchymal injury[37].

Anitha Uthandi et al observed higher levels of serum AST, ALT and ALP of high fat fed rats compared to control groups indicates the hepato toxic role of high fat diet[38]. Similar results were found in our study. An elevation in the levels of these enzymes is generally considered as one of the most sensitive index of the hepatic damage[38].

Increased levels of serum ALP levels in rats fed with high fat diet clearly indicates disturbed excretory functions of liver which coincide with present study[39].

As enzymes SGOT and SGPT are mainly present in the hepatic and biliary cells. Elevated levels of these enzymes in serum of high fat fed rats shows hepatocellular damage caused due to high fat toxicity[40].

Anitha Uthandi et al reported decreased levels of serum protein in rats fed with high fat diet are possible due to localized damage in the endoplasmic reticulum[38]. The fruit of *Emblica Officinalis* is a considered as major constituent of liver tonic used against acute viral hepatitis and various liver disorders[41].

Rupal A Vasant et al observed significantly reduced plasma ALP levels in the rats treated with Amla which supports our observation[42].

V Damodar Reddy reported that after administration *of Emblica Officinalis* fruit extract to rats treated to alcohol improved the histomorphology of the liver near to normal[43]. The *Emblica Officinalis* fruit extract supplementation to rats in our study clearly indicate the hepatoprotective role also preserves the structural integrity of the liver from the adverse effects of alcohol.

6.7: Kidney functions:

High fat diet causes up regulation of fatty acids with increased triglyceride synthesis due to impaired triglyceride transportation. It leads to fat accumulation in the renal tissue. Renal steatosis is associated with high levels of visceral adiposity and adipokines which causes renal dysfunction. Chronic inflammation leads to lipid accumulation in the glomeruli and proximal tubules of kidneys along with increased expression of markers[44].

High fat diet can alter balance between lipogenesis and lipolysis in the kidney, hence causing renal lipid accumulation and lipid Peroxidation. Subsequently it leads to endothelial dysfunction and over expression of inflammatory factors, fibrosis and oxidative stress[30].

Kume et al reported that mice on high fat diet have shown insulin resistance at four weeks, renal steatosis at eight weeks and renal injury at sixteen weeks. Renal damage is directly related to continuous stress and insulin resistance[45].

Due to increased adipose mass and microphage infiltration causes renal hypertrophy. Increase in renal volumes of high fat fed rats is related to inflammation, vasodilatation, connective tissue enlargement and renal damage[46].

Renal dysfunction is associated with abnormal regulation of RAAS throughout the kidney. Angiotensin II is found only limited areas of kidney and linked with intra renal RAAS regulation. Angiotensin II has been related to dyslipidemia, insulin resistance which may cause obesity related renal diseases. Altered levels of angiotensin can cause renal damage[47].

Castro et al observed that rats fed with high fat diet showed central obesity, increased heart rate and renal inflammatory infiltrates although serum creatinine and urea levels were unchanged[48]. It was also demonstrated that the young HFD-fed rats exhibited central obesity, increased blood pressure, increased heart rate, and renal inflammatory infiltrates, despite serum creatinine and urea levels remaining unchanged[48].

High fat diet produces alterations in renal lipid metabolism because of imbalance between lipogenesis and lipolysis in the kidneys. Subsequently there will be accumulation of lipid in renal tissue and renal damage[49].

Kidney functions from our study clearly depicts that, high fat fed rats have shown significant increase in serum creatinine and urea levels and *Emblica Officinalis* (Amla) have shown a protective action against lipid induced altered kidney functions. It is possibly due to presence of high flavonoids and polyphenolic compounds with its antioxidant property act as renoprotective agent.

Y S Bhalodia et al observed significant increase in serum creatinine and urea levels indicating glomerular dysfunction in rats fed with high fat diet with ischemic reperfusion[50].

Manish K Singh et al reported significant reduction in creatinine levels in mice supplemented with amla compared to mice exposed to arsenic[41].

It has been also mentioned that oral administration of Amla for 28 days in STZ-induced diabetic rats reduces blood glucose level, creatinine and urea levels in serum and reduced oxidative stress by increasing levels of antioxidant enzymes, thus, ameliorates kidney dysfunction in diabetes rats and these observation are similar as in our study[51].

Treatment with EEO in our study demonstrated clearly its reno protetctive effect against dietary insult of high fat by attenuating the severity of renal pathological damage and improving renal functions in our study[52].

6.8: Oxidative and Nitrosative stress:

Swaraj Bandhu Kesh et al mentioned that high fat diet cause dyslipidemia related oxidative stress by accumulating free radicals. Subsequently it leads to increased expression of NADPH oxidase and reduced expression of antioxidant enzymes[53]. Lipid peroxidation and ROS can ingest antioxidant enzymes and vitamins[54]. Decrease in these protective substances cause ROS inactivation and increase lipid peroxidation and ROS-mediated damage[55]. This subsequently activate one of the major transcription factors linked with inflammation such as NF-κB, inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF-α) interferon γ (IFN-γ)[56]. Moreover overproduction of nitric oxide (NO) by activated iNOS; in turn excess generation of reactive nitrogen species (RNS) or pyroxinitrite are imminent due to the interaction between superoxides and NO[53].

A decrease in SOD activity contributes to increasing the level of superoxide radicals, thus leading to increased oxidative stress. Further decrease in catalase activity would increase H_2O_2 concentration in the cell, leading to increased lipid peroxidation and oxidative stress[57].

Glutathione reductase reduces the oxidized form of Glutathione to GSH. A decrease in the activity of glutathione reductase would further decrease the concentration of ascorbic acid. Our finding of the decreased activity of superoxide dismutase, catalase, GSH corroborates with that of earlier findings[57]. It has been well documented from various studies that amla is a powerful antioxidant. *Emblica officinalis* reduced the oxidative stress by its free radical scavenging property. This antioxidant potential has been attributed to flavonoid contents of *Emblica offficinalis*[57]. It has also been noted that phenolic content in ethanolic extract of amla has shown antioxidant potential by inhibiting auto-oxidation via free radical scavenging, singlet oxygen quenching and hydrogen donating mechanisms[58].

Javed Ansari et al reported obesity induced oxidative stress in rats fed with high fat diet. Similar observations were observed in our study. Our results have shown that after supplementation of EEO to hyperlipidemic rats have shown significant increase in the activity of superoxide-dismutase, catalase, glutathione and ascorbic acid [59].

Vitamin C present in the extract of Amla maintains the first natural antioxidant defense activity and acts as a powerful inhibitor of lipid peroxidation[57]. Possibly, emblicanin A of EEO aggressively seeks and attacks free radicals. After it neutralizes a free radical, Emblicanin A is transformed into Emblicanin B, another antioxidant. Emblicanin B in turn attacks free radicals and is transformed into emblicanin oligomers thus prevent further oxidative damages[57].

Supplementation of aqueous extract of *Emblica officinalis* (Amla) reduced erythrocyte malondialdehyde levels in type 2 rats indicating that Amla could be a potent inhibitor of

oxidative damage in erythrocytes[59]. Supplementation of hydro alcoholic extraction of Amla produced higher levels of anti‑oxidant enzymes; which may result in reduction of hydrogen peroxides and protect the tissues from highly reactive hydroxyl[2].

It was documented that in rats fed with high fat diet established inactivity of NO bioactivity, alterations of endothelial nitric oxide synthase (eNOS) or NOS3 and inducible nitric oxide synthase (iNOS) or NOS2. These changes are supported by downregulation of eNOS expression in aorta, as well as overexpression of iNOS protein in vascular tissues[60].

Subsequently ellagic acid and ascorbic acid in extracts of Amla found to accelerate antioxidant property by increase in nitric oxide and decrease in hydroxyl radicals through its free radical scavenging property and prevention of LDL oxidation in our study[61, 62]. Unaltered NOS3 protein with decreased NO in high fat fed rats of our study indicate a possible alteration of vascular pathophysiology probably through oxygen sensing cell signaling pathway[17].

6.9: Glucose homeostasis:

High fat diet supplementation to the rats causes increased plasma FFA with decreased glucose uptake, glycolysis, glycogen synthesis. Enhanced availability and oxidation of FFA lead to impaired glucose metabolism. Chronic HF is also known to increase -oxidation in animal models and inhibition of -oxidation increases insulin-stimulated glucose uptake[63].

Normally under physiological conditions adipose tissue can buffer fatty acid flux into circulation by avoiding excess exposure to lipotoxic stimuli. It is well associated with suppression of FFA and TG release also increased clearance of circulating TG. Eventually all these three mechanisms are hampered in experimental animals fed with high fat diet. Higher levels of TG are due to excess rate of intestinal lipid absorption, changed lipid handling and/or lower insulin sensitivity of the adipose tissue[64].

Noemi A V Roja et al reported that high fat fed rats showed less number of relative islet area and inclined to have reduced beta cell area causing impaired beta cell replication[49]. Limited beta cell reserve in rats fed with high fat diet is sufficient to maintain fasting glucose and insulin levels but is not sufficient to preserve glucose stimulated insulin secretion and glucose tolerance[49]. Diabetes and dyslipidemia are two main factors which revolve around the pathophysiological effects of abnormal lipid levels and insulin resistance[65]. Excess FFA storage from abnormal lipid metabolism leads to insulin resistance in peripheral cells, eventually causing hyperinsulinemia, hyperglycemia along with hyperlipidemia. However diabetes mellitus develops through pre diabetic state and land up in diabetic state if dietary lipid is not well regulated[66]. Hence retaining blood glucose homeostasis efficiently, has become indispensible concern in prevention of diabetes mellitus.

High fat diet is associated with insulin resistance and reduced insulin secretion by beta cells in the pancreas which may lead to altered glucose homeostasis in rats fed with high fat diet in our study[66]. The possible link between hyperlipidemea and beta cell dysfunction of pancreas may be due to elevated plasma Free Fatty Acids (FFA). Lipotoxicity caused by elevation of FFA induces diabetogenic effect[67]. Our results on glucose concentration indicate alteration of insulin secretary mechanism rather than insulin resistance. Possibly autonomic malfunction observed from our results on LF/HF altered beta cell function in rats fed with HFD.

Creg et al. (2016) reported in their study that after 6 weeks of hyperlipidemic, hypercholestremic diet, rats were presented with dyslipidemia, hyperinsulinemia and elevated levels of TG[68]. These elevated TG, increase glucose production, and acts on peripheral action of insulin. It was reported that rats fed with high fat diet for 12 weeks developed insulin resistance along with increased levels of cholesterol[69]. Increased blood glucose, decreased plasma insulin

with higher HOMA-IR in high fat fed rats clearly indicates an alteration of glucose homeostasis in our study[70]. HOMA index was shown to be increased in rats fed with high fat diet indicating insulin-resistance development in these animals as previously reported by[70] *Emblica officinalis* supplementation is effective in reducing blood glucose levels by regenerating and rejuvenating beta cells of pancreas and increasing insulin production and secretion[4].

Our observation on EEO supplementation to HFD fed rats (Group 4) showing a reduction in fasting and postprandial blood glucose levels supports the earlier study[71]. The decrease in TC and TG levels with supplementation of *Emblica officinalis* in our study hypothesizes that a possible inhibition of lipolysis in adipose tissue due to insulin sensitizing or insulin mimetic effect of polyphenolic compound of *Emblica officinalis*[2].

6.10: Histopathology

6.10.1: Myocardium

Figure no 5.13.1 we observe microscopic architecture of myocardium did not show any significant change of myocardial tissue in group 3 rats, except we observed in few rats myocardium containing coronary artery showing early changes of atherosclerosis which indicate minimal cardiac metabolic disturbances by high dietary fat[72].

6.10.2: Elastic artery:

Histopathological assessment of elastic artery wall showing in the endothelial layer an early change of atherosclerotic plaque and even we observe that there is a mild alteration (thickening) in the arterial wall histological architecture. These alterations in present study may include arterial wall modification with component changes in the arterial wall and same in stiffer aorta.

In our study early atherosclerotic changes in group 3 rats reflect less elasticity in arterial wall and may lead to increase in peripheral resistance and blood pressure. The result indicates a possible arterial stiffness due to high dietary fat intake[73]. 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 arteries[74].

Increase thickness in the wall 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 hyperlipidemic group in our study[75]. 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 tension[76]. Figure no 5.13.2, we have clearly found decreased arterial lumen, increased wall thickness which again bring back to the normal in case of groups 4 and 5 with supplementation of drugs *Emblica Officinalis* (Amla) and Statin.

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[77] Thus, alteration of mechanical priority which may lead to severe cardiovascular dysfunction[76]. 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[78]. In our study the supplementation of *Emblica Officinalis* (Amla*)* and statin 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 shows that improvement of elastic arterial property by treatment with *Emblica Officinalis*(Amla) (group 4) and Statin (group 5) hence EEO is found to be beneficial against high dietary fat induced alteration of vascular pathophysiology

6.10.3: LIVER

Liver is the major organ of metabolic and energy homeostasis. Its balanced actions are over levels of endogenous metabolites such as TG, TC, HDL and glucose[79]. The

hepatoprotective actions of *Embilica Officinalis* noticed to be mediated by its free radical scavenging, antioxidant and modulation of lipid metabolism[80]

Hyperlipidemic rats liver microscopic architecture showed the lobular architecture with enlarged Hapatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion. Administration of hyperlipidemic diet with *Emblica Officinalis* showed near normal appearance of hepatocytes[81]

In the present study we tried to rule out the effect of *Embilica Officinalis* on pathophysiology of liver of hyperlipidemic rats Figure no 5.13.3, Histopathology of liver in control rats have shown normal hepatic architecture compressed of hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation but whereas group 2 rats fed with (Amla), group 4 hyperlipidemic rats treated with & 5 has shown prominent sinusoidal dilatation. Group 3 rats have shown lobular architecture of the liver with enlarged hepatocytes containing microvescicular and macrovesicular fatty changes with sinusoidal congestion.

6.10.4: KIDNEY

The nephroprotective effect of Ethanolic extract of *Embilica Officinalis* (Amla) to prevent the development of renal dysfunction and alteration of histopathology of kidney which are assessed by biochemical and renal markers in a hyperlipidemic rat model. Hyperlipidemic animal models express changes in renal markers, biochemical parameters and histopathology of kidney. As *Emblica officinalis* being potent antioxidant exerts free radical scavenging activity and shows preventive role against fat induced renal toxicity[82] Pretreatment with Amla extract

demonstrated its nephroprotective effect by attenuating the severity of renal pathological damage and improving renal functioning[52].

Figure no 5.13.4 we have observed that histopathology of renal corpuscles exhibited normal appearance in groups 1 and 2 whereas in group 3 showed prominent increase in the glomerular capillaries and also renal architecture exhibiting the focal glomerular lesions, including thickening of glomerulus and completely decreased space between glomerulus and Bowmen's capsule. Whereas group 4 and 5 showing mild alterations of architecture of kidney with normal renal corpuscles

REFERENCES:

1) Govind P, Pandey SP. Phytochemical and toxicity study of Emblica officinallis (Amla). Int Res. J Pharma. 2011;2(3):270-2.

2) Patel SS, Goyal RK et al. Experimental study on effect of hydroalcoholic extract of Emblica Officinalis fruits on glucose homeostasis and metabolic parameters. Ayu 34(4); 2013 Oct; 34(4):440.

3) Hasan MR, Islam MN, Islam MR. Phytochemistry, pharmacological activities and traditional uses of *Emblica Officinalis*: A review. International Current Pharmaceutical Journal 2016 January ; (2):14-21.

4) Picchi MG, Mattos AM, Barbosa MR, Duarte CP, Gandini MD, Portari GV. A high fat diet as a model of fatty liver disease in rats. Acta Cir Bras 2011; 26 (2):25-30.

5) Das KK, Dasgupta S. Effects of nickel sulfate on testicular streogenesis in rats during protein restriction. Environ Health perspect 2012; 110(9):923-26.

6) Badea AR, Nedelcu L, Valeanu M, Zdrenghea D. the relationship between seru lipid farctions and heart rate variability in diabetic patients with statin therapy. Clujul Medical 2014;87(3):152-58.

7) Poirier P, Hernandez TL, Weil KM, Shepard TJ, Eckel RH. Impact of diet induced weight loss on the cardiac autonomic nervous system in severe obesity. Obesity 2003 Sep1;11(9):1040-7.

8) Fu Ch, Yang CC, Lin CL, Kuo TB. Alteration of cardiovascular autonomic functions by vegetarian diets in postmenopausal women is related to LDL cholesterol levels. Chinese Journal of Physiology 2008 Apr 30; 51(2):100

9) Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141:122-31.

10) Chaar LJ, Coelho A, Silva NM, Festuccia WL, Antunes VR. High fat diet induced hypertension and autonomic imbalance are associated with upregulation of CART in the dorsomedial hypothalamus of mice. Physiological reports 2016 Jun1; 4(11):12811.

11) Roy A, Kundu D, Mandal T, Bandyopadhyay U, Ghosh E, Ray D. A comparative study of heat rate variability tests and lipid profile in healthy young adult males and females. Nigerian Journal of Clinical Practice 2013; 16(4):424-28.

12) Crege DRXO, Miotto AM, Borghi F F, Wolf-NunesnV, Kassisse DMG. Cardiometaabolic alterations in wister rats on six-week hyperlipidic, hypercholesterolemic diet. International Journal of Cardiovascular Sciences 2016; 29(5):362-69.

13) Kepez A, Dogan Z, Mammadov C, et al. Evaluation of association between hyperlipidemia and heart rate variability in subjects without apparent cardiovascular disease. Kosuyolu Heart Journal 201; 18(1):29-33.

14) Pehlivanidis AN, Athyros VG, Demitriadis DS, Papageorgiou AA, Bouloukos VJ, Kontopoulos AG. Heart rate variability after long term treatment with atorvastatin in hypercholestrolaemic patients with or without coronary artery disease. Atherosclerosis 2001; 157: 463-9.

15) Da Silva Termarin C, Casali KR, Meurer L, Schaan BD. Capsaicin induced metabolic and cardiovascular autonomic improvement in an animal model of the metabolic syndrome. british Journal of Nutrition 2014 Jan;111(2):207-14.

16) Khaliq F, Parveena A, Fahim M. Effect of Terminalia Arjuna on heart rate variability in diabetic rats. Int. J. Curr. Res. Chem. Pharma. Sci.2014;1(5):44-47.

17) Das KK, Chadchan KS, Reddy RC et al. effects of some indigenous plants of North Karnataka (India) on cardiovascular and glucose regulatory systems in diabetic rats. Cardiovasc Hematol Agents Med Chem 2017;15 (1):49-61

18) Cortez M, Carmo LS, Rogero MM, Borelli P, Fock RA. High fat diet increases IL-1, IL-6 and TNF production by NF-kB and attenuating PPAR-γ expression in bone marrow mesenchymal stem cells. Inflammation 2013 Apr 1; 36(2):379-86.

19) Trottier MD, Naaz A, Li Y, Fraker PJ. Enhancement of homeostasis and lymphopoeisis in diet induced obese mice. Proceedings of the National Academy of Sciences 2012 May 15;109 (20):7622-9.

20) Adler BJ, Green DE, Pagnotti GM, Chan ME, Rubin CT. High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche. Plos one 2014 Mar 4; 9(3): e90639.

21) Arumugam Shivagurunathan et al. Immunoctimulatory potential of dietary Amla (Phylanthus Emblica) in growth and haematology of Tilapia mossambicus challenged with pseudomonas aeruginosa. International research Journal of Pharmacy 2012; 3(7):16-68.

22) Raj CD, Jayanthi V, Manaswini VS, Gayatri R, Ranjani C, Brindha P. Effect of polyherbal formulation on high fat diet induced hyperlipidemia in rats. International Journal of Pharmacy and Pharmaceutical Sciences 2012;4(2):31-35.

23) Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non alcoholic fatty liver disease. Journal of gastroenterology 2013 Apr 1;18(4):434-41.

24) Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and non alcoholic fatty liver disease. International Journal of molecular Sciences 2014 Apr 11;1(4):6184-223.

25) Verges B. Pathophysioloy of diabeteic dyslipidaemia: where are we? Diabetologia 2015 May 1:58(5):886-99.

26) Nimmanpalli HD, Kasi AD, Kumar Devapatla P, Nuttakki V. Lipid ratios, atherogenic coefficient and atherogenic index of plasm as parameters in assessing cardiovascular risk in thype 2 diabetes mellitus. International Journal of research in medical sciences 2017bJan 3; 4(7):2863-9.

27) Walia K, Boolchandani R, Dhand S, Antony B. improving glycaemic and lipidemic profile with amla powder (Emblica Officinalis) supplementation in adults with type 2 diabetes mellitus. IJBAMS 2015; 5(2):251-58.

28) Mann S, Beedie C, Jimenez A. Differential effects of aerobic exercise, resistance training and combined excerise modalities on cholesterol and the lipid profile: review, synthesis and recommendation. Sports medicine 2014; 44(2):211-21.

29) Patel SS, Goyal RK. Prevention of diabetes induced myocardial dysfunction in rats using the juice of the Emblica Officinalis fruit. Experimental & Clinical Cardiology 2011;16(3):87-91.

30) Ruster C, Wolf G. Adipokines promote chronic kidney disease. Nephrol Dial Transplant 2013;28(4):8-14.

31) Klein S. The case of visceral fat: argument for the defense. Journal of Clinical investigation 2004 Jun 1;113 (11):1530.

32) Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab 2008;93:57-63.

33) Kakimoto PA, Kowaltowski AJ. Effects of high fat diets on rodent liver bioenergetics and oxidative imbalance. Redox Biology 2016 Aug 31; 8:216-25.

34) Byrne CD. Fatty liver: role of inflammation and fatty acid nutrition, prostaglandins leukot.Essent.Fat.Acids 2010;82:265-71.

35) Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis 2015; 239:192-202.

36) Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of non alcoholic steato hepatitis. Free Radic.Biol.Med. 2012;52:59-69.

37) Mazumdar S, Mukherjee S, Mitra A, Karmarkar S, Das AS, Mukherjee M. Folic acid or combined of folic acid and vitamin B12 prevent short term arsenic trioxide induced systemic and mitochondrial dysfunction and DNA damage. Environ Biophys 2009;85:860-77.

38) Uthandi A, Ramasamy K. Hepatoptotective activity of sesame meal on hih fat fed wister rats. Int J Pharm Sci res 2011;2(12):205-11.

39) Kothavade RJ, Joylekar SN, Barodavalla SA. Protective effect of indigenous drug livomyn on ketoconazole induced hepatotoxicity. Indian J Pharm Sci 1996;58:142-46.

40) Norman JJ. Common Laboratory Test in liver diseases. Columbia-edu. Columbia 1998.

41) Singh MK, Dwivedi S. Arsenic induced hepatic toxixcity and its attenuation by fruit extract of Emblica Officinalis(Amla) in mice. Ind J Clin Biochem 2014 Jan-Mar;29(1):29-37.

42) Vasant RA, A.V.R.L. Narasimhacharya. Amla as an antihyperglycemic and hepato-renal protective agent in fluoride induced toxicity. Journal of Pharmacy and Bio allied sciences 2012;4(3):250-4.

43) Reddy VD, Padmawathi P, Gopi S, Paramhansa M, Varadacharyulu NC. Protective effect of Emblica Officinalis against alcohol induced hepatic injury by ameliorating oxidative stress in rats. Indian Journal of Clinical Biochemistry 2010 Oct1;25(4):419-24.

44) Stemmer K, Perez TD, Ananthkrishnan G, et al. High fat diet induced obesity causes an inflammation and tumor promoting microenvironment in the rat kidney. Dis Model mech 2012; 5:627-35.

45) Kume S, Uzu T,Araki S,et al. role of altered renal lipid metabolism in the development of renal induced by a high fat diet. J Am Soc Nephrol 2007;18:2715-2723.

46) Altunkanyak ME, Ozebek E, Alyunkanyak BZ, Can I, Unal D, Unal B. The effects of high fat diet on the renal structure and Morphometric parametric of kidneys in rats. J Anat 2008; 212:845-52.

47) Deji N, Kume S, Araki S, et al. Structural and functional changes in the kidneys of high fat diet induced obese mice. Am J Physiol Renal Physiol 2009:118-126.

48) De Castro, Uberdan GM, Santos D,etal. Age dependent effect of high fructose and high fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids Health Dis 2013;12:136.

49) Roza NA, Possingnolo LF, Palanch AC, Gontijo JA. Effect of long term high fat diet on peripheral insulin sensibility, blood pressure and renal function in female rats. Food & Nutrition rsearch 2016 Jan 1;60(1):28536.

50) Bhalodia YS, Patel NJ, Patel RK, Vaghasiya JD, Jivani NP, sheth NR. Benincasa cerifera ameliorates renal iscaemia/reperfusion injury in hyperlipidemic rat. Pharmacognosy Research 2009 Nov 1;1(6):406.

51) Thakkar NV, Patel JA. Pharmacological evaluation of 'Glyoherb': A polyherbal formulation on strptozotocin induced diabetic rats. International Journal of diabetes in developing countries 2010; 30(1):1-7.

52) Tasanarong A, Kongkham S, Itharat A. Antioxidant effect of phyllanthus Emblica extract prevents contrast induced acute kidney injury. BMC Complementary and Alternative Medicine 2014;14:138.

53) Kesh SB, Sarkar D, Manna K. High fat diet induced oxidative stress and its impact on metabolic syndrome: A review. Asian J Pharm Clin Res 2016;9(1):47-52.

54) Pessayre D, Mansouri A, Fromenty B. Non-alcoholic steatosis and steatohepatitis. Mitochondrial dysfunction in steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2002; 282(2):G193-99.

55) Le Lay S, Simard G, Martinez MC, Andriantsitohaina R. Oxidative stress and metabolic pathologies: from an adipocentric point of view. Oxid Med Cell Longev 2014;2014:90839.

56) Weisberg SP, Leibel R, Tortoriello DV. Dietry Curcumin significantly improves obesity associated inflammation and diabetes in mouse models of diabesity. Endocrionology 2008; 149(7):3549-58.

57) Chakraborti D, Verma R. Ameliorating effect of Emblica Officinalis aqueous on ochratoxin induced lipid Peroxidation in the kidney and liver of mice. International Journal of occupational medicine and environment health 2010; 23(1):63-73.

58) Sultana S, Ahmed S, Khan N, Jahangir T. effects of Emblica Officinalis(Gaertn) on CCl4 induced hepatic toxicity and DNA synthesis of wister rats. Indian J Exp Biol 2005;43:430-6.

59) Ansari JA, Bhandari U, Pillai KK, Haque SE. Effect of rosuvastatin on obesity induced cardiac oxidative stress in wistar rats-a preliminary study. Indian Journal of Experimental Biology 2012; 50:216-222.

60) Pakdeechote P, Bunbupha S, Kukongviriyapan U, Prachaney P, Khrisanapant W, Kukongviriyapan. Asiatic acid alleviates hemodynamic and metabolic alterations via restoring eNOS/iNOS expression, oxidative stress and inflammation in diet induced metabolic syndrome rats. Nutrients 2014; 6(1):355-70.

61) Kaur J, Kaur D, Singh H, Khan MU. Emblica Officinalis; a meritocratic drug for treating various disorders. Indo American Journal of Pharmaceutical Research 2013;3(6).

62) Naampoothiri SV, Prathapan A, Cherian OL, Raghu KG, Venugopalan VV, Sundaresan A. A in vitro antioxidant and inhibitory potential of Terminalia bellica and Emblica Officinalis fruits against LDL oxidation and key enzymes linked to type 2 diabetes. Food and chemical Toxicol 2011;49(1):125-31.

63) Park SY, Kim YW, Kim JY, Jang EC, Doh KO, Lee SK. Effect of high fat diet on insulin resistance :dietary fat versus visceral fat mass. Journal of Korean medical Science 2001;16(4):386.

64) Hernandez EA, Kahl S, Seeling A, et al. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. The journal of Clinical investigation 2017 Feb;127(2):695-8.

65) David S, Paul Z. Diabetes and hyperlipidemia: Adirect quantitative analysis of the effects of insulin resistance on lipid levels in relation to atherosclerotic coronary artery disease. WJCD 2012; 2:20-25.

66) Chang WC, Wu JS, Chen CW. Protective effect of Vanillic acid against hyperinsulinemia, hyperglycemia and hyperlipidemia via alleviating hepatic insulin resistance and inflammation in high fat diet (HFD) fed rats. Nutrients 2015;7(12):9946-59.

67) Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW. Direct effect of Cholesterol on insulin secretion. Diabetes 2007; 56(9):2328-38.

68) Crege DRXO, Mitto AM, Borghi F Wolf Nunes V, Kassisse DMG. Cardiometabolic alterations in wister rats on a six week hyperlipidemic, hypercholesterolemic diet. Int J Cardiovas Sci 2016; 29(5):362-69.

69) Apaijai N, Pintana H, Chattipakorn SC, Chattipakorn N. Cardioprotective effe ts of metformin and vidagliptin in adults rats with insulin resistance induced by a high fat diet. Endocrinology 2012; 153(8):3878-85.

70) Nascimento TB, Baptista RD, Pereira PC, etal. Vascular alterations in high fat diet obese rats:role of endothelial L-arginine/NO pathway. Arquivos brasileiros de cardiologia 2011Jul; 97(1):40-5.

71) Akhtar MS, Ramzan A, Ali A, Ahmad M. Effect of amla fruit(Emblica Officinalis Gaertn) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. Int J Food Sci Nutri 2011; 62(6):609-16.

72) Haurington J, Pana, AS, Gent R, Hirtec, Courter J. Aortic intima media thicknes in an early marker of atherosclerosis in children with type I diabeties mellitus. Journal of Pedator. 2010 ; 156 : 237- 41

73) O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D. Clinical applications of arterial stiffness; definitions and reference values. American journal of hypertension 2002 May 1;15(5):426-44.

74) Sentelices L C, Rutaman S J, Ertart J C, pranti RL, hanay J N, Vorp D A and Aharan J M . Experimental system for ex vivo measurement of Murine aortic stiffness. Physiological measurement. 2007; 28 (8): 39-49.

75) Haurington J, Pana, A S, Gent, R. Hirtec, Couter J. Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes mellitus. Journal of Pedator. 2010; 156: 237-41.

76) Billaud M, Johnstone SR, Isakson BE. Loss of compliance in small arteries, but not in conduit arteries, after 6 weeks exposure to high fat diet. Journal of cardiovascular translational research. 2012 Jun 1; 5(3):256-63.

77) Deopujari R, Dixit A. The study of age related changes in coronary arteries and its relevance to the atherosclerosis. J Anat Soc India. 2010 Dec 1; 59(2):192-6.

78) Rizzoni D, D Ciuceis, C. Porteri E, Semerarao F and Rosri E A. Structural alterations in small resistance arteries in obesity. Basic and Clinical pharmacology and toxicology. 2011; 1742-1843.

79) Režen T, Tamasi V, Lövgren-Sandblom A, Björkhem I, Meyer UA, Rozman D. Effect of CAR activation on selected metabolic pathways in normal and hyperlipidemic mouse livers. BMC genomics. 2009 Aug 19;10(1):384.

80)Thilakchand KR, Mathai RT, Simon P, Ravi RT, Baliga-Rao MP, Baliga MS. Hepatoprotective properties of the Indian gooseberry (Emblica officinalis Gaertn): a review. Food & function. 2013; 4(10):1431-41.

81) Patil BS, Desai SD, Potekar RM. Effect of ethanolic extract of *Emblica Officinalis* (*amla*) on pathophysiology of liver in hyperlipidemic albino wister rats. Int J Pharm Bio Sci 2015 July; 6(3): (B) 362-72.

82) Patil BS, Desai SD, Kanthe PS et al. Effect of ethanolic extract of *Emblica Officinalis* on histopathology of kidney and on biochemical parameters in hyperlipidimic albino rats. JKIMSU 2015;4 (3).

CHAPTER 7

SUMMARY AND CONCLUSION

SUMMARY & CONCLUSION:

The present study on the effect of high fat diet (30%) on intermediary metabolism of albino rats with special reference to drug *Emblica Officinalis* (Amla) can be summarized as following in the graphical abstract below, (Figure 7.1)

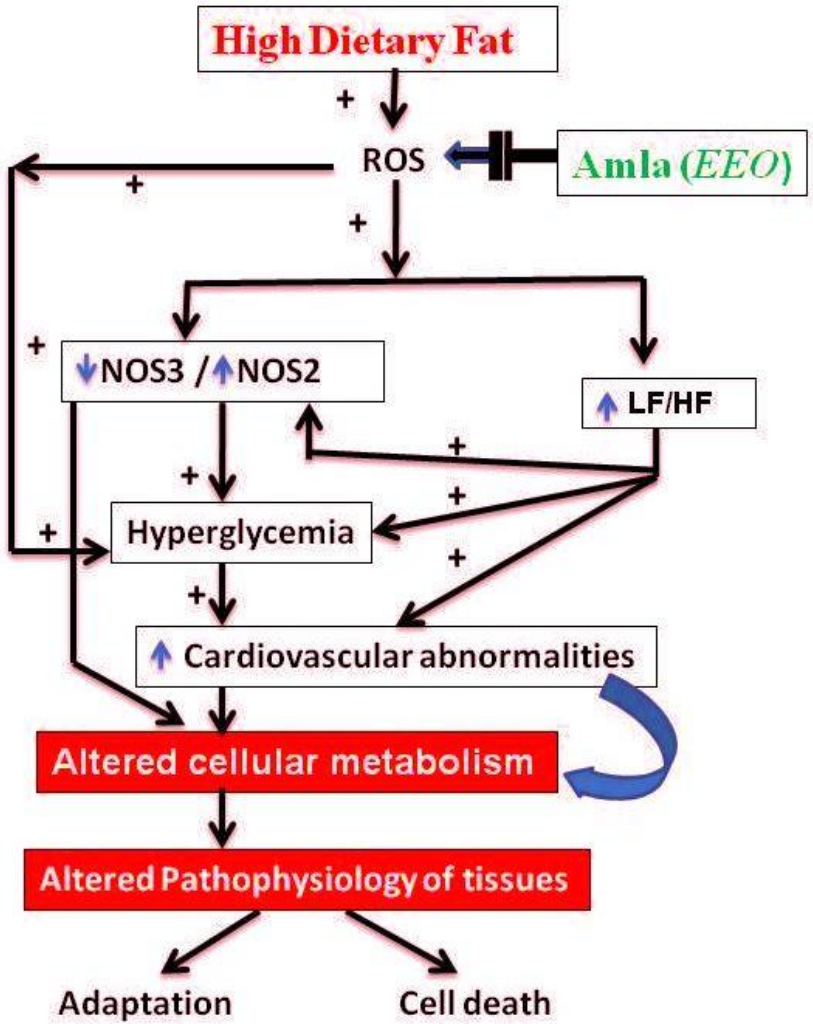


Figure 7.1: Graphical abstract

It can be concluded that,

- 10% of extra fat to control recommended dietary fat (20%) i.e. 30% of total fat, sub chronic exposure (21 days) in adult rats develops alterations of intermediary metabolism like dyslipidemia, hyperglycemia and disturbed hepato-renal functions.

- High fat diet can induce cardiac autonomic malfunctions in albino rats.
- High fat diet possibly causes oxidant- antioxidant imbalance in albino rats.
- High fat diet induces altered pathophysiology of metabolically active tissues and vascular architecture.
- This is further corroborated with histopathological study on liver, kidney, myocardium and elastic artery.
- *Emblica Officinalis* contains many biological compounds like tannins, gallic acids, flavonoids etc which posses medicinal properties. Probably polyphenolic compounds and flavonoids of EEO might have the protective role by various means including antioxidant activities, lipid regulations and glucose homeostasis in high fat fed experimental animals.
- Ethanolic extract of *Emblica officinalis* supplementation found to be beneficial against high fat induced dyslipidemia and altered glucose metabolism.
- Ethanolic extract of *Emblica Officinalis* (Amla) might have cardio protective actions in high fat fed rats by modulating cardiac autonomic functions.
- Supplementation of EEO as antioxidant ameliorates fat induced oxidative stress in metabolically active tissues which further protects intermediary metabolism and cardiovascular health.

Limitation and Future direction

Study limitations: Present study on *Emblica Officinalis* was done with phytochemical extract only but active biological compounds and possible synthetic derivatives were not done.

Future prospective: Synthesis of derivatives from EEO and target these compounds for treatment of ailments connected to hyperlipidemea or glucose regulatory mechanisms as form of herbal medicine. A thorough study on bioactive compounds or its synthetic analogue in cell signaling pathways especially on vascular pathophysiology induced by hyperlipidemea may be explored.

INSTITUTIONAL ANIMAL ETHICAL CLEARENCE

BLDEU'S DEPT. OF PHARMACOLOGY VIJAYAPUR-586103 OUTWARD No. 664/15 Date: 7/12/15 SHRI B.M. PATIL MEDICAL COLLEGE

Chairman,
Institutional Animal Ethics Committee (IAEC),
Prof. & HOD, Dept. of Pharmacology,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

Dr. Pallavi Kanthe
Ph.D Student in Physiology,
BLDEU's Shri. B.M.Patil Medical College,
BIJAPUR.

ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 07.12.2015 at 10.30am to scrutinize the Research Project submitted by faculty member of this College.

After scrutiny the following research project has been accorded ethical clearance.
Title: "**Effect of ethanolic extract of *Emblica Officinalis* (AMLA) on intermediary metabolism of albino rats fed with hyperlipidemic diet**"
Principal investigator: Dr. Kanthe Pallavi, PhD Student in Physiology.

07.12.2015

Dr. R. S. Wali
Chairman, (IAEC).
Professor & HOD
Dept. of Pharmacology
BLDEU's Shri B. M. Patil
Medical College, VIJAYAPUR.

16.1.16

PAPER PRESENTATIONS

Paper presentations at conferences:

Oral presentations:

At National conference:

1) Title: *Emblica officinalis* (Amla): its effect on pathophysiology of liver in Albino Rats fed with hyperlipidemic diet.

3rd ASSOPICON 14th -17th September 2016, 'Physiology Decodes Novelty of Vascular Sciences' at BLDEU's Shri B M Patil medical college, Hospital & Research Centre, Bijapur.

Received **"Best paper Award"**

2) Title: Effect of Ethanolic extract of *Emblica Officinalis* on intermediary metabolism and liver histopathology of rats fed with hyperlipidemic diet.
64th NATCON, 28th Nov-1st Dec , 2016 at AIIMS Jodhpur, Rajasthan.

At International conference:

1) Title: Evaluate the effect of ethanolic extract of *Emblica Officinalis* (amla) on histopathological alterations of kidney and biochemical parameters in hyperlipidemic albino rats.

19th KCA CON, World congress of Anatomists, GIMS, Gadag, 22nd to 25th September 2017.

Poster presentation:

At international conference:

1) Title: Protective effects of Ethanolic extract of *Emblica Officinalis* (amla) on cardiovascular pathophysiology in hyperlipidemic rats.

FIPSPHYSIOCON 2017, on 'Integrating Physiological and biomedical Science Approaches to improve performance, health and safety'. At Defence Institute of Physiology & Allied Sciences, New Delhi, 5th -7th November, 2017.

PUBLICATIONS

1. Kanthe PS, Patil BS, Bagali SC, Reddy RC, Aithala MR, Das KK. Protective effects of Ethanolic Extract of *Emblica officinalis* (amla) on Cardiovascular Pathophysiology of Rats, Fed with High Fat Diet. Journal of Clinical and Diagnostic Research : JCDR. 2017;11(9):CC05-CC09. **(Indexed in Pubmed)**
2. Kanthe PS, Patil BS, Aithala MR, Das KK. Effect of Ethanolic Extract of *Emblica officinalis* (Amla) on Glucose Homeostasis in Rats Fed with High Fat Diet. JKIMSU2017; 6(3). **(Indexed in Scopus)**
3. Das KK, Chadchan KS, Reddy RC et al. effects of some indigenous plants of North Karnataka (India) on cardiovascular and glucose regulatory systems in diabetic rats. Cardiovasc Hematol Agents Med Chem 2017;15 (1):49-61**(Indexed in Pubmed)**