CHAPTER-V

INTRODUCTION

Obesity is becoming one of the greatest threats to global health in this century. It is a multifactorial disease termed as chronic disorder of carbohydrate and lipid metabolism and is characterized by an increased fat deposition in adipose tissue and other internal organs. Obesity is also associated with lifestyle changes, inadequate dietary habits, low basal metabolic rate, less physical activity and low fat oxidation. In the case of Obesity condition the body mass index (BMI) is $>30$ kg/m$^2$. To reduce the body mass index, physical activity plays a crucial role. Physical activity is helpful in the prevention of obesity by increasing the basal metabolic rate. Obesity is significantly associated with development of many diseases like type 2 diabetes, cardiovascular disease, certain cancers respiratory difficulties, chronic musculoskeletal problems, lumbago, skin problems, and infertility (Guh et al., 2009). Obesity is not only developed as a result of dietary and lifestyle factors it is also developed by genetic influences. Osteoporosis is another one important cause for obesity. Obesity is not only caused due to increased adipose tissue but also caused due to chronic inflammation and it can be characterized by macrophage infiltration (Wellen and Hotamisligil, 2003). Relationship between the body mass index and bone mass index has been reported by various researchers. Lot of studies proved that adipose tissue protects the skeleton (Clark et al., 2006). Visceral adipose tissue is inversely correlated with bone and subcutaneous adipose tissue is directly related to bone size and its density (Gilsanz et al., 2009).

WHO estimated that obesity in 2011 has doubled as compared to the population in 1980’s. The prevalence of obesity is increasing globally, approximately half a billion of world population is considered as overweight or obese (Rossner, 2002). In 2014 more than 1.9 billion adults become overweight and out of these 600 million were obese. Prevalence of obesity in India is up to 50% in women and 32.2% among men in the upper society. Obesity is controlled by appetite regulation and energy metabolism. Physical inactivity is increased in our lives, hence it decreases the energy expenditure and increases the energy uptake. Appetite regulation is also maintained by hormone like leptin that is synthesised in the adipose tissue.
**Adipose tissue**

Adipose tissue is an endocrine organ, which is involved in the synthesis of adipokines and cytokines. Adipocyte secreting adipokines are involved in many metabolic pathways to express its function. An association exists between obesity and chronic inflammation through release of cytokines like interleukin (IL)-1β, IL-6, TNF-α and monocyte chemo-attractant protein (MCP)-1.

**Adipocyte secreting proteins**

The adipocyte secreted proteins like adiponectin, leptin, resistin, apelin, interleukins and tumor necrosis factor-α, are crucial for energy homeostasis of multiple physiological processes (Avram et al., 2007; Gregoire, 2001).

**Tumor Necrosis Factor Alpha (TNF-α)**

TNF-α is a cytokine involved in the systemic inflammatory response and is also associated with insulin resistance, obesity and diabetes. It is produced mainly by monocytes, lymphocytes, adipose tissue, and muscle, and also it’s irregular production leads to the pathogenesis of the obesity-associated metabolic syndrome.

**Interleukin**

Interleukin is a cytokine that exerts many effects like defence, inflammation and tissue damage. Interleukin is produced both by macrophages and adipocytes and by immune system cells, fibroblasts, endothelial cells and skeletal muscles. Circulating levels of IL-6 correlate with BMI, insulin resistance and intolerance to carbohydrates. IL-6 also influences glucose tolerance through negative regulation of visfatin along with the secretion of adiponectin. It causes elevation of TG levels by enhancing gluconeogenesis and glycogenolysis and inhibiting glycogenesis.

**Adipsin**

Adipsin is a serine protease that is secreted by adipocytes and is related to adiposity, insulin resistance, dyslipidemia and cardio vascular diseases. Adipsin is regulates fatty acid uptake by adipose tissue and then convert it into triacyl glycerol.
**Resistin**

Resistin is an adipokine that is produced by adipocytes and macrophages. It has link with obesity and insulin resistance. Resistin is encoded by the RETN gene. Resistin play a crucial role in energy homeostasis.

**Leptin**

Leptin has 166 amino acids and is the product of human obese (OB) gene located on chromosome 7. Leptin is synthesised in the adipose tissue and also synthesised in small amounts in stomach, heart, mammary epithelium and placenta. Leptin acts via the leptin receptor (LEPR or OBR). Leptin is the product of OBR gene located on chromosome 1p31 and is highly expressed in hypothalamus and cerebellum. After secretion of leptin by the adipose tissue it enters into the brain via the blood stream. Leptin is involved in the regulation of food intake and energy balance. Leptin is secreted from white adipose tissue and its levels in the circulation are correlated with the degree of adiposity (Loftus, 1999).

**Multiple leptin signaling pathways (Figure 5.1)**

Leptin plays an important role in energy intake and energy expenditure. Leptin facilitates its effects by binding to leptin receptors (LepRs) expressed in the brain and peripheral tissues by alternatively spliced isoforms of LepR. LepRb is involved in leptin signaling. LepRb is expressed in the hypothalamus, where it regulates energy homeostasis, food intake, neuroendocrine function, memory and learning. If there is a deficiency in leptin and LepRb leads to development of hyperphagia, morbid obesity, infertility and reduced linear growth (Kelesidis et al., 2010). Hyperphagia and morbid obesity is caused due to leptin deficiency or loss of function due to mutations of the leptin receptor in humans. If leptin bind to the receptor LepRb activates a number of signaling pathways JAK2/STAT 3 and STAT5, IRS/PI3K, SHP2/MAPK, and AMPK/ACC. The leptin signaling cascade is terminated by the induction of a suppressor of cytokine signaling 3 (SOCS3).

**SOCS3 inhibits JAK2/STAT3 signaling**

Suppressor of cytokine signaling3 (SOCS3) inhibits JAK2/STAT3 signaling, providing a negative feedback mechanism. In addition to which protein tyrosine phosphatase 1B (PTP1B) is implicated in the negative regulation of leptin signaling.
Obese individuals have elevated adipose leptin expression and plasma leptin levels with high concentration leads to excess adiposity.

The binding of leptin to leptin receptor (LepRb) results in dimerization, leading to the formation of the LepRb/Janus kinase 2 (JAK2) complex. The activated JAK2 phosphorylates Tyr985, Tyr1077 and Tyr1138 in LepRb. Signal transducer and activator of transcription (STAT) 3 and STAT5 bind to phospho-Tyr1138 and phospho-Tyr1077 in LepRb are then phosphorylated. Active STAT3 and STAT5 dimers then translocate to the nucleus and activate the transcription of their target genes, which mediate leptin’s anorexigenic effect. The Suppressor of cytokine signaling 3 (SOCS3) is a target gene of STAT3, inhibits the JAK2/STAT3 pathway by interacting with phospho-Tyr985 or JAK2 and acting as a feedback inhibitor of leptin signaling. Protein tyrosine phosphatase 1B (PTP1B) also inhibits leptin signaling through dephosphorylation of
JAK2. After JAK2 activation, SH2-containing protein tyrosine phosphatase 2 (SHP2) binds to phospho-Tyr985 in the LepRb and the adaptor protein growth factor receptor bound protein 2 (Grb2), leading to the activation of mitogen-activated protein kinase (MAPK) signaling cascade. Leptin activates MAPK independent of SHP2 and also regulates phosphatidylinositol 3 kinase (PI3K) signaling through insulin receptor substrate (IRS) phosphorylation. Forkhead box O1 (FoxO1), mammalian target of rapamycin (mTOR), and phosphodiesterase 3B (PDE3B) are important downstream targets of PI3K in the leptin signaling pathway.

**Leptin and AMPK**

Leptin inhibits feeding and metabolism in the brain and also the peripheral organs. Leptin activates 5’-AMP-activated protein kinase (AMPK) in muscle, inhibiting acetyl coenzyme A, an enzyme that catalyzes a key step in fat synthesis. As a result, the energy sources that are involved in the formation of fat, then enter into an oxidative pathway by providing energy for the muscle cells. So leptin inhibits the food intake.

**Energy homeostasis and leptin signaling**

Leptin is more commonly expressed in the hypothalamus, mainly in the arcuate nucleus(ARC) and ventromedial hypothalamus(VMH). Leptin directly targets two neuronal populations in the ARC co-expressing proopiocortin(POMC) and amphetamine regulated transcript(CART), agouti-related peptide(AgRP) and neuropeptide Y (NPY) (Xu et al., 2011). Leptin stimulates POMC/CART expression and inhibits AgRP/NPY expression, thereby reducing food intake, increasing energy expenditure and decreasing body weight. In addition, leptin inhibits feeding by reducing the expression of melanin-concentrating hormone (MCH) and orexins in the lateral hypothalamic area (LHA). Leptin also stimulates the expression of brain-derived neurotrophic factor and steroidogenic factor-1 (SF-1) neurons in the VMH, leading to inhibition of feeding (Morris and Rui 2009, Kim et al., 2011).

The binding of leptin to LepRb activates JAK2, and the activated JAK2 phosphorylates Tyr985, Tyr1077, and Tyr1138 in the cytoplasmic domain of LepRb. If mutation occurs in these three tyrosines in LepRb induces obesity in mice but to a lesser degree than in LepRb-deficient, this indicating that LepRb affects energy homeostasis through both tyrosine-dependent and independent mechanisms. Leptin’s activation of JAK2/STAT3 signaling appears to play a major role in energy homeostasis and neuroendocrine function. Once phosphorylated, STAT3 is translocated from the
cytoplasm into the nucleus, where it binds to POMC and AgRP promoters, stimulating POMC expression and inhibiting AgRP. Indeed, deletion of STAT3 in neurons decreases POMC and increases AgRP and NPY levels, ending in hyperphagia, obesity, infertility, and thermal dysregulation (Gao et al., 2004). Moreover, a specific deletion of Tyr1138 in LepRb or STAT3 in LepRb-expressing neurons results in hyperphagia and morbid obesity (Bates et al., 2003, Piper et al., 2008). STAT5 deletion in the brain develop hyperphagia and obesity but less severely than in mice with STAT3 deletion, indicating that JAK2/STAT5 plays a minor role in leptin’s regulation of feeding and body weight (Lee et al., 2008).

The extracellular signal related kinase (ERK), a member of the MAPK family, acts downstream of LepRb. Activation of ERK1/2 by either SHP2 from Tyr985 of LepRb or directly from JAK2. Neuron-specific deletion of SHP2 results in obesity and leptin resistance in mice (Zhang et al., 2004). Pharmacological blockage of hypothalamic ERK1/2 abrogates the anorectic and weight-reducing effects of leptin in rats. Inhibition of ERK also prevents leptin-induced sympathetic activation of brown adipose tissue, indicating that SHP2/MAPK signaling is involved in leptin regulation of food intake and energy expenditure (Rahmouni et al., 2009).

Adiponectin

Adiponectin is a polypeptide hormone containing 247 amino acids and is discovered in the year 1995 (Trujillo and Scherer, 2005; Hu et al., 1996). Adiponectin is a protein synthesized in adipose tissue. Adiponectin is also called as GBP-28, apM1, AdipoQ and Acrp30. Adiponectin is a protein which is encoded by the gene ADIPOQ gene. Adiponectin play an important role in the glucose regulation and also in fatty acid breakdown.

The adiponectin receptors like adipoR1 and adipoR2 have been identified and found to be expressed in various tissues (Kadowaki and Yamauchi 2005). AdipoR1 is abundantly expressed in skeletal muscles, whereas adipoR2 is present predominantly in the liver, suggesting a role of adipoR2 in hepatic adiponectin signaling (Woo et al., 2012; Kadowaki et al., 2008). Adipo R1 and Adipo R2 receptor is important for adiponectin protein in the regulation of glucose and lipid metabolism.

Mechanism of Adiponectin

Adiponectin stimulates AMPK pathway in different tissues like skeletal muscle, liver, heart, endothelium, adipocytes and brain. Adiponectin is a protein that bind to the
receptor, an adaptor protein containing a pleckstrin homology domain, a phosphotyrosine binding domain and a leucine zipper motif, which appears to be the key signaling molecule that couples adiponectin receptors and its downstream AMPK activation (Deepa and Dong 2009). AMPK activation in turn phosphorylates acetyl coenzyme A carboxylase (ACC) and attenuates ACC activity. Inhibition of acetyl CoA carboxylase inhibits the lipid synthesis and enhances fatty acid oxidation by inhibiting the production of malonyl CoA by allosteric inhibitor of carnitine palmitoyl transferase I, which is the rate limiting enzyme in fatty acid oxidation. The activation of AMPK downregulates the expression of sterol regulatory element binding protein 1c (SREBP1c), a transcription factor that regulates cholesterol and lipid synthesis. Reduction of SREBP1c results in downregulation of genes involved in lipogenesis, including ACC, fatty acid synthase, and glycerol-3-phosphate acyltransferase (Yamauchi et al., 2002). The mechanism is explained in Figure 5.2.

PPARα is a transcription factor controlling the transcription of genes encoding fatty acid oxidation enzymes, such as FATP, acyl-CoA oxidase and long chain acyl-

Figure 5.2: Mechanism of Adiponectin

Source: dx.doi.org
CoA synthetase. Adiponectin stimulates PPARα activity possibly through PPARγ coactivator-1α (You and Rogers, 2009). This adiponectin activated signaling pathways leads to fat oxidation and reduce the lipid synthesis.

**Lipid metabolism and causes**

Lipid metabolism is maintained by both lipid synthesis and degradation. The abnormalities in lipid metabolism leads to the development of hyperlipidemia and hypercholesterolemia. This can causes diseases like obesity, diabetes, arthrosclerosis, hypertension and functional depression of some organs (Goldstein et al., 1973) 3-hydroxy 3 methyl glutaryl coA reductase (HMG CoA) is an enzyme involved in the synthesis of lipids. Pancreatic lipase is an enzyme involved in the degradation of lipids to fatty acids (Rodwell et al., 1973).

**Importance of Dietary Models**

There are a number of commercially available diets that can produce obesity in certain susceptible rodents. Animals that gain excessive weight on the special diets are considered to be genetically susceptible, whereas those which are resistant to weight gain are considered to be genetically resistant (Levin et al., 1987).

Dietary models are the powerful tool for elucidating the environmental and genetic factors that cause obesity. The dietary models of obesity include the high fat diet used by Dobrian et al., 2000. The high-fat and carbohydrate diets are generally used to induce obesity. These models tend to show the importance of oxidative stress (Dobrian et al., 2001).

Deng et al., 2007 reported that high cholesterol or high fructose diet induce obesity. They reported that these diet decrease insulin resistance and deterioration of myocardial contractility in rats. The results showed significant lipid accumulation in the myocardium, left ventricular hypertrophy and morphological liver damage. In rats the combination of high-cholesterol and high fructose diet leads to an increase of cholesterol plasma levels, decrease of HDL-cholesterol and increases the weight of the liver.

**Diet induced obesity**

High carbohydrate diet and high fat diet leads to adiposity.
Obesity induced by high carbohydrate diet

High carbohydrate diets are associated with increase body mass and also increase the circulating triglycerides and develop the insulin resistance in humans and animals. Many researchers reported the relationship between fat intake and obesity in mice and rats showed similar results.

Nemoseck et al., 2011 reported that sucrose induces body weight gain and evaluated the insulin, lipid profile, leptin and adiponectin levels by comparing the weight of the gonadal fat as compared with the control diet group.

Kanazawa et al., 2003 showed the gain in body weight with sucrose feeding which leads to plasma triglycerides and stress tolerance in rats. Feeding with sucrose diet (60%) for two weeks did not induce higher body weight gain compared to the standard diet. In the present study using 30% sucrose from the second week there was a greater weight gain in the sucrose group compared to control (standard diet). The diet increased plasma triglyceride level in lean and obese mice.

Obesity induced by high fat diet

Diet that contains high fat and carbohydrate causes obesity, if intake of fat rich foods, which are associated with high energy intake (Little et al., 2007). The foods with high energy and high dietary fat content promote increase in fat storage and weight gain in humans. Fat diets that have 9kcal/g are compared with 4kcal/g for carbohydrates and proteins (Schrauwen et al., 2000). High fat diet is a factor for developing hyperglycemia and hyperinsulinemia. Highest intake of refined sweet carbohydrates (fast food, desserts) promotes weight gain, visceral adiposity and diseases related to obesity. High-fat diet (HFD)-induced obesity in animal models is believed to be best and mimic the physiological functions of an obese body.

High fat diet may leads to triglycerides (TAG) accumulation in liver and also import excess amount of fatty acids into the liver. The excess of fatty acids are then esterified and stored as triglycerides. Triglyceride accumulation is also caused due to methionine or choline deficient diet, which further induces a reduction in VLDL synthesis and finally results in hepatic steatosis. (Kirsch et al., 2003; Lombardi et al., 1968; Powell et al., 2005). The hepatic import of plasma glucose and synthesis of fatty acids are increased during fasting condition. During this process, β-oxidation of fatty acids is inhibited. Also, in a fasted state, the concentrations of circulating glucose and insulin are low, which stimulates the degradation of TAG into fatty acids in adipose
tissue. The free fatty acids are absorbed in the liver and degraded via β-oxidation, and ketone bodies are synthesized and are used for energy shortages. In the liver the triglyceride synthesis is inhibited and free fatty acid exceeds the hepatic lipolytic capability and it leads to excess fatty acids which are stored in the liver as TAG. Hepatic TAG increases with fasting in mice (Lin et al., 2005).

The degradation and synthesis, import and export of triacylglycerol and fatty acids is balanced by hepatic lipid metabolism. Liver incorporates plasma glucose and fatty acids. Glucose is degraded by the glycolytic system, and the acetyl CoA produced by this system is used for the fatty acid synthesis. Fatty acids are degraded via β-oxidation, or esterified and then stored as TAG. Triacyl glycerol is degraded into fatty acids and glycerol in the liver, or directly exported to the extrahepatic circulation as very-low density lipoprotein (VLDL) (Bender and Mayes, 2006).

Based on the fat content present in the diet the high fat diet is divided into low fat diet (10%), high fat diet (30-50%). If high fat diet exceeds 50% it is consider as very high fat diet.

Kim et al., 2000 reported that increased content of triacylglycerol in muscle by HFD induction was responsible for the insulin resistance.

Buettner et al., 2006 studied the metabolic and molecular effects of olive oil, cocoa oil, lard and cod liver oil using HFD. They found that highest level of obesity, insulin resistance and hepatic steatosis as well as activation of SREBP1c (sterol response element-binding protein 1c). The SREBP is a transcriptional regulation of hepatic synthesis of fatty acids, resulting due to lard ingestion.

Liver enzymes

Cellular damage is caused due to liver enzymes in serum. Liver enzymes such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), Gamma gluramyl transaminase (GGT) and alkaline phosphatase (ALP) and protein parameters likes total protein, albumin and globulin in humans and animals act as indicators of liver injury. SGOT and SGPT are sensitive indicators of acute hepatic necrosis (Hussain et al., 2012).

High fat diet increases the cholesterol content exogenously by the activities of SGOT and SGPT. The activity of Lactate dehydrogenase tends to increase due to the presence of hyperlipidemia with high lipid and blocking caused by the accumulation of lipids in liver tissue.
Hepatic fat accumulation leads to the imbalance between lipid uptake and lipid clearance. The mechanism of triglycerides, fatty acids accumulation in adipose is the major source of lipids in the steatotic liver. Fatty liver associated with obesity is an independent risk factor for liver peroxidation which results in cell damage and elevated levels of serum enzymes. Liver damage is prevented by antioxidant capacity. High fat diet depresses the antioxidant system due to increased lipid peroxidation and the formation of free radicals.

**High fat diet induces hepatosteatosis (Figure 5.3)**

Obesity is commonly associated with insulin resistance, which are the risk factors for type 2 diabetes, cardiovascular disease, hepatic steatosis and non-alcoholic steatohepatitis (Browning and Horton, 2004). Excess of triglycerides are not only stored in adipocytes and excess of lipids stored in liver disturbs insulin signaling (Goossens, 2008). Visceral fat mass is associated with insulin resistance and non-alcoholic fatty liver disease (NAFLD) (Calamita and Portincasa, 2007). Visceral fat released from free fatty acids are transported to liver by the portal vein and are involved in the formation of hepatic steatosis and formation of triglyceride rich very low density lipoproteins (VLDL) (Jensen, 2008).

**Production of ROS during hepatic steatosis**

Hypercholesterolemia, hyperglycemia and hypertension are the sources of increased oxidant stress in the obese state (Vincent and Taylor, 2006). Koboyasi et al., 2010 stated that the production of superoxide anion and the Cu/ZnSOD activity increased in obese mice. Nakao et al., 2000 also reported significant increase in SOD levels in the liver, kidney, muscles, plasma and white and brown adipose tissues of obese mice. Vincent et al., 2001 found that the Cu/ZnSOD activity in the left ventricles of rats showed higher level in the obese animals compared to lean controls. Superoxide dismutase activity was associated with obesity (Erdev et al., 2004).

**Fatty liver**

Fatty liver is a condition which causes the liver fibrosis subsequently. The production of excess of reactive oxygen species (ROS), lipid peroxidation, leads to the release of inflammatory cytokines, death of hepatocytes and activation of hepatic stellate cells. ROS and lipid peroxidation are causes of the liver injury leading to inflammation caused due to increased ROS production (Browning and Horton, 2004). ROS production is reduced in hepatocytes by adiponectin via activation of PPARα.
(Neumeier et al., 2006). Adiponectin also increases ROS detoxifying enzymes and AdipoR2 receptor is involved in the induction of superoxide dismutase 1 and catalase (Yamauchi et al., 2007).

Reactive oxygen species can disrupt the normal metabolism and physiology. This condition can lead to the onset of health disorders and have major damaging role in liver disorders (Favier, 2006). ROS production is increased during oxidative stress that occurs during hepatic steatosis.

**Oxidative stress**

Oxidative stress is termed as imbalance between oxidant and antioxidant levels leading to potential cellular damage. Normal cells can with stand mild oxidative stress, as they have sufficient antioxidant defence mechanism and repair systems, which play a crucial role in removal of molecular damages by oxidation. The imbalance can result from an overabundance of reactive oxygen species (ROS). ROS as potential carcinogen play an important role to damage the lipids, protein or DNA and inhibiting normal function if it is not regulated properly. ROS alterations in different signaling pathways may modulate gene expression, cell adhesion, cell metabolism, cell cycle and cell death.

**Lipid peroxidation**

Lipid peroxidation is defined as “the oxidative deterioration of polyunsaturated lipids” (Halliwell and Gutteridge, 1999). Lipoproteins and fatty acids are the targets of oxidative stress. Lipid peroxidation causes the attack on the hydrogen atom from methylene group. Lipid peroxidation is mainly caused in membranes that surround the cells. The biological membranes contain lipids like lecithin, cholesterol and phospholipids. Peroxidation of linoleic acids leads to two hydroperoxides. Peroxidation of arachidonic acid will produce six lipid hydroperoxides as well as cyclic peroxides and other products, including the isoprostanes. Dietary antioxidants, like α-tocopherol and β-carotene, can prevent lipid peroxidation through mechanisms such as scavenging or quenching (Sies, 1992).

Lipid peroxidation mediated tissue damage has been observed in the development of hyperlipidemia (Stan Kubow et al., 1996). Increased levels of lipid peroxidation in hyperlipidemic condition causes the increased oxidative stress in cells as a result of depletion of antioxidant scavenging systems. Inflammation related to various infection results in increased lipid peroxidation with decreased antioxidant levels. Which can further leads to oxidant-mediated injury in the liver (Favier, 2006).
Enzymatic and Non-Enzymatic Antioxidants

Obesity is associated with the oxidant stress increase, even in early age. The obesity-associated oxidant stress include increased oxygen consumption and subsequent radical production via mitochondrial respiration, increased fat deposition and cell injury causing increased rates of radical formation (Vincent et al., 2001).

Cells have different types of antioxidant mechanism and also various antioxidant enzymes act against the free radical damage. Glutathione peroxidase (GPx) is a selenoprotein, which reduces lipidic or non-lipidic hydroperoxides, as well as H₂O₂ while oxidizing Glutathione (GSH). Normal differentiation is caused due to the alteration in enzymatic systems. Glutathione peroxidase is the enzyme that effectively reduces lipid hydro peroxides within the biological membranes (Herrera and Barbas, 2001; Packer et al., 2001).

Catalase

Catalase is an enzyme which is present mainly in the peroxisomes of mammalian cells. It is a tetrameric enzyme consisting of four identical, tetrahedral subunits of 60 kDa, each containing heme group and NADPH in its active center. Depending on the concentration of H₂O₂, catalase has different enzymatic activities. If the concentration of H₂O₂ is high, catalase acts catalytically, and removes H₂O₂ by forming H₂O and O₂. Catalase acts peroxidically, removing H₂O₂, but oxidizing its substrate (peroxidatic reaction) (Scibior and Czeczot, 2006).

Superoxide dismutase

Superoxide dismutase activity is one of the major enzymatic antioxidant mechanisms against superoxide radical, in preventing liver toxicity induced by oxidative stress (Yagmurca et al., 2007). Catalase and Glutathione peroxidase was observed to catalyze dismutation of the superoxide anion (O₂⁻) into hydrogen peroxide (H₂O₂) which then converts H₂O₂ to water thus providing protection against ROS. Superoxide dismutase (SOD) and catalase (CAT) prevent reactive oxygen metabolites and protect the cell from cellular and molecular damage (Aebi, 1984).

The superoxide dismutases (SOD) are ubiquitous components of cellular antioxidant systems. Superoxide dismutases are typically soluble secreted or cytosolic proteins but are also found in a number of subcellular compartments such as the cell envelope of gram-negative bacteria or the mitochondria of eukaryotic cells. Superoxide
radicals can damage the membrane and the biological structure. Superoxide dismutase (SOD) can protect the tissues against oxygen free radicals by the removal of superoxide radical. Reduced activities of SOD and CAT in the kidney have been observed during hyperlipidemia and this may result in the accumulation of superoxide and hydrogen peroxide radicals (Pedraza-Chaverri et al., 2000).

The non-enzymatic antioxidant includes vitamin C which acts as a cytosolic antioxidant, Vitamin E act as a membrane antioxidant against free radical attack. Vitamin C is otherwise called as ascorbic acid. Ascorbic acid is the most important antioxidant in extra cellular fluids and has many cellular activities. Vitamin C is highly effective antioxidant. It acts as substrate for ascorbate peroxidase. Vitamin E is a fat-soluble antioxidant which is the major antioxidant found in lipid-phase membranes. It decrease the production of ROS. When fat undergoes oxidation, mitochondrial damage is prevented by vitamin E.

Ascorbic acid

Ascorbic acid or vitamin C is a monosaccharide antioxidant found in both animals and plants. Ascorbic acid is a reducing agent that can reduce and thereby neutralize, reactive oxygen species such as hydrogen peroxide (Ortega, 2006). Ascorbic acid in the cell is maintained in its reduced form by reaction with glutathione.

Vitamin E

Tocopherols and tocotrienols (vitamin E) are fat-soluble vitamins with antioxidant properties. The tocopherol is the most important lipid-soluble antioxidant and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction.

Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister and Anderson, 1983).
Apolipoproteins

Lipids are transported by lipoproteins present in the blood. Apolipoproteins or apoproteins are the polypeptide found in various types of lipoproteins. Lipoproteins are three major groups like Apo A, Apo B and Apo C and also Apo D, E, H and J types are characterized by various physiological and chromosomal locations. The apolipoprotein genes are located on chromosome 1, 2, 3, 6, 11 and 19. Apolipoproteins are involved in the transport of chylomicrons, triglyceride, cholesterol and fatty acids.

Apolipoprotein B is divided into two type Apo B-100 and Apo B-48. Apo B 100 is produced in liver and present in lipoproteins like very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL). Apo B 48 is produced in the intestine and found in chylomicrons and their remnants. Apolipoprotein B is found in chromosome 2. Apo B-48 has 2152 amino acids and Apo-B-100 has 4536 amino acids.

Apolipoprotein B (Apo B) is the protein part of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), Low density lipoprotein (LDL) and play a crucial role in lipoprotein metabolism. Apolipoprotein B is involved in the production and secretion of VLDL and also acts as a ligand for the LDL receptors. So it helps in the removal of LDL particles from the plasma and in the transport of cholesterol to peripheral cells and reverses to the liver. Abnormal metabolism of Apo B is responsible for the generation of hyperlipidemia and is also associated with the risk of developing coronary heart disease.

Homocysteine

Homocysteine is formed from the essential amino acid methionine. The metabolism of homocysteine is trans-sulfuration or re-methylation. During re-methylation pathway of homocysteine to methionine, Vitamin B12 and folate act as cofactors (Fowler, 1997) in the presence of the enzyme methylene tetra hydro folate reductase (Goyette et al., 1994).

Homocysteine and dyslipidemia

Homocysteine (Hcy) and lipids are toxic in vascular cells and hepatocytes. The interactions between the two pathways develop hepatic steatosis and atherosclerotic lesions. The PPARα expression is lower in case of homocysteinemia. Decreased expression of PPARα is also responsible for increased expression of cholesterol 7α-hydroxylase. This enzyme is rate limiting enzyme in cholesterol to bile acids formation.
Increased expression of cholesterol 7α-hydroxylase enhances cholesterol absorption in the intestine that result in bile acids production which is increased in the liver.

The sterol regulatory element binding protein (SREBP-1) is an endoplasmic membrane bound transcription factor that activate the genes encoding enzymes in the cholesterol or triglyceride biosynthesis and uptake pathways (Horton and Shimomura, 1999). Hyperhomocysteinemia enhance the expression of SREBP and also enhances the accumulation of cholesterol (Werstruck et al., 2001). Homocysteine activate the unfolded protein response genes in endoplasmic reticulum which can accelerate SREBP-1 activity. This increases the synthesis of cholesterol and triglycerides.

**Pancreatic lipase**

Pancreatic lipase is synthesised from pancreas, which plays an important role in the digestion of dietary fat. Pancreatic lipase catalyses the breakdown of triglycerides into monoacylglycerol and free fatty acids. Decreasing the lipid absorption is one of the mechanism of antiobesity effects. Pancreatic lipase inhibitory effect is one of the most important factors for determining the efficacy of antiobesity agent. Orlistat is the potent inhibitor of pancreatic lipase which is used for the treatment of obesity.

**Orlistat**

Orlistat (tetrahydro lipostatin) is a hydrogenated derivative of lipstatin derived from *streptomyces toxitricini*, is a potent inhibitor of pancreatic, carboxyl ester and gastric lipase and has been proved to be effective for the treatment of obesity (Ballinger and Peikin, 2002). Orlistat is otherwise called as Xenical. In 1999 orlistat was approved by the FDA for the management of obesity. Orlistat is a class of antiobesity agents that inhibit gastrointestinal lipases. The structure of orlistat was shown in Figure 5.4.

**Pharmacology:**

Orlistat is a potent inhibitor of intestinal lipases. Orlistat inhibits 46.6% to 91.4% intestinal lipase activity. Intestinal lipases are responsible for the breakdown of dietary triglycerides into fatty acids and monoglycerides and then absorbed by mucosal cells. If non absorbing triglycerides levels are increased in the faeces, it inhibits the lipase activity, while orlistat reduces fat absorption by approximately 30%. During circulation of orlistat reduced amount of fat is absorbed. Orlistat undergoes hydrolysis at the β-lactone ring to form different metabolites that are excreted in the bile. Orlistat can be
used along with cyclosporine because it inhibits the organ rejection during transplant. Hence cyclosporine is used as an immunosuppressive agent.

Orlistat is clinically approved as a drug for the treatment of obesity, but it has some side effects in gastrointestinal tract, oily spotting, liquid stools and fecal urgency (Chaput et al., 2007). Due to this it has adverse effects and there is a demand for the development of nontoxic and lipid reducing product from natural sources.

Hence current chapter was focussed on the effect of ethanolic extract of Benincasa hispida fruit extract (EEBH) and active fraction of Benincasa hispida (AFBH) in high fat diet (HFD) induced rats.

**Figure 5.3: High fat diet induce hepatic steatosis**
**Materials and methods**

**Selection of Animals and Ethics**

Healthy male Wistar albino rats weighing about 200-210g were obtained from Siddha Central Research Institute, Arumbakkam, Chennai - 600 106. Animals were housed in cages under proper environmental conditions at room temperature 22-24°C for 12 h light/dark cycle and fed with a commercial pellet diet and water *ad-libitum*. The animals had free access to water. The animals were acclimatized to the laboratory conditions for two weeks before beginning the experiment. The experiment continued for 8 weeks on which constant weight of the diet was given for animals. All the experiments were designed and conducted according to the Control and Supervision of Experiments on Animals [CPCSEA], Ministry of Social Justice and Empowerment, Government of India. [135/PHARMA/SCRI.2013]. CPCSEA guidelines for laboratory animal facility were followed.

**Preparation of Plant Material**

The fresh fruit extract of *Benincasa hispida* was collected from medicinal plant vendor. The ethanolic extract of *Benincasa hispida* were prepared as mentioned in chapter 1 and active fractions was collected using column chromatography as discussed in chapter 3. The ethanolic extract of *Benincasa hispida* (EEBH) and active fraction of *Benincasa hispida* (AFBH) was used for this study.
High fat diet

Rats were housed in polypropylene cages with free access to drinking water and food. They were fed standard rat chow, that was procured from Tamil Nadu Veterinary and animal Sciences University, Kattupakkam, Chennai and animals were fed with HFD. HFD composition is as follows,

High fat diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gm%</th>
<th>Kcal%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>26.2</td>
<td>20</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>26.3</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>34.9</td>
<td>60</td>
</tr>
<tr>
<td>Total(Kcal/gm)</td>
<td>5.24</td>
<td>100</td>
</tr>
</tbody>
</table>

The energy given by the normal diet is 3.43 Kcal/g and the HFD is 5.24 Kcal/g. High fat diet contain 34.9 % fat.

Ingredients of High fat diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gm%</th>
<th>Kcal%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Corn starch</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>Sucrose</td>
<td>68.8</td>
<td>275.2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>25</td>
<td>225</td>
</tr>
<tr>
<td>Lard</td>
<td>245</td>
<td>2205</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>16.5</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Choline Bitartarate</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Induction of Experimental Animal and treatment protocol

The animals were grouped into five groups of six rats in each group.

**Group I:** Control - rats fed with standard diet.

**Group II:** High fat diet fed rats for 8 weeks.

**Group III:** HFD fed rats were treated with 300mg/kg of ethanolic extract of *Benincasa hispida* fruit extract (EEBH) orally for 8 weeks.

**Group IV:** HFD rats were treated with 100mg/kg of active fraction IV of ethanolic extract of *Benincasa hispida* fruit extract (AFBH) orally for 8 weeks.

**Group V:** HFD rats were treated with 25mg/kg of orlistat for 8 weeks.

**Standard**

Orlistat used as standard anti-obesity drug and was purchased from Sigma Aldrich.

During the experimental periods body weight changes, food intake and water intake was noted daily.

**Measurement of Body Weight and Body Weight Gain**

Body weight was measured throughout the experimental period. The body weights of different groups of rats were weighed for each week and body weight gain (BWG) was calculated by the formula as,

\[ \text{Body Weight Gain} = \text{Final weight (g)} - \text{Initial weight (g)}. \]

**Measurement of Food Intake**

Food Intake was measured once per day at a fixed time. Fresh water was supplied every day at the same time. Their weights were assessed with an electrical weighing balance. The weights of food consumption were expressed in grams.

**Anthropometrical Determinations**

Anthropometrical determinations were made in anaesthetized rats by the method of Novelli *et al.,* 2007. The anthropometrical parameters were expressed in centimetres. The abdominal circumference (AC) was anterior to the forefoot, thoracic circumference (TC) was behind the foreleg, body length from nose to anus and weight of animals was determined. Body weight and length of the rats were used to determine the body mass index.

\[
\text{Body Mass Index (BMI)} = \frac{\text{Body weight (g)}}{\text{Length}^2 \text{ (cm)}}
\]
Body fat pad determination

The fat pad weight was measured. Fat pad such as perirenal, mesenteric, epididymal and gonadal fat pad was measured after sacrifice of the animals.

Collection of Serum

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in control and experimental animals. After the experimental period the animals were sacrificed by cervical decapitation. The blood was collected by retro orbital artery bleeding. Blood sample was centrifuged for 10 minutes at 3000 rpm to separate the serum. This serum was used for further studies. Plasma was used for homocysteine analysis.

Determination of Biochemical Parameters

Estimation of Cholesterol

Serum cholesterol was estimated by Zak’s method (Zlatkis et al, 1953) Ferric chloride reagent precipitates the cholesterol. Cholesterol reacts with concentrated sulphuric acid to form pink colour complex which is read at 540 nm. The intensity of pink coloured complex is directly proportional to the concentration of cholesterol in serum.

Reagents

★ Ferric chloride reagent: 0.05% ferric chloride in glacial acetic acid (aldehyde free).
★ Concentrated sulphuric acid.
★ Cholesterol stock standard: 200 mg of cholesterol dissolved in 100 ml acetic acid and 4 ml of stock is diluted with 100 ml of ferric chloride acetic acid reagent, (80 µg/ml)

Procedure

0.1 ml of serum was added to 9.9 ml of ferric chloride reagent in a glass stoppered centrifuge tube for the preparation of protein free filtrate. The content was mixed and allowed to stand for 20-30 minutes. To 5 ml of the protein free filtrate, 3ml of concentrated sulphuric acid was added and mixed by swirling. Standards were also treated similarly. Blank contains ferric chloride acetic acid reagent with concentrated sulphuric acid. All the test tubes were allowed to stand for 20 minutes and intensity of the colour developed was read at 540 nm. The values are expressed as mg/dl of serum.
**Estimation of Serum Triglycerides**

Triglycerides in serum were estimated by the method of Rice, 1970.

**Reagents**

- **Isopropanol**
- **Activated alumina:** 10 g of aluminium oxide was taken in a beaker and 30 ml of distilled water was added. The suspension was stirred well and the supernatant was discarded. The suspension was washed with distilled water and the process was repeated several times until the supernatant becomes clear. The water was discarded and aluminium oxide was activated in an oven at 90°C overnight. The dried powder was then used for the analysis.
- **Alkaline potassium hydroxide:** 10 g of potassium hydroxide was dissolved in 75 ml of distilled water and 25 ml of isopropanol was added to the solution.
- **Sodium metaperiodate reagent:** 7.7 g of anhydrous ammonium acetate was dissolved in 70 ml of distilled water. To this 6 ml of acetic acid was added followed by 65 mg of sodium metaperiodate. The solution was mixed well and made up to 100 ml with distilled water.
- **Acetyl acetone reagent:** 0.4 ml of redistilled acetyl acetone was added to 100 ml of isopropanol and mixed well. It was stored in the refrigerator.
- **Standard triglyceride solution:** Stock Solution: 1 ml of glycerol trioleate was dissolved and made up to 100 ml with isopropanol.

**Procedure**

To 0.1 ml of serum, 3.9 ml of isopropanol was added followed by 50 mg of activated alumina. It was mixed well and left for 15 minutes. It was then centrifuged and 2 ml of the supernatant was taken for analysis. 6 ml of alkaline potassium hydroxide was added to all the tubes. The tubes were incubated at 60°C for 10 minutes. The tubes were cooled and 1 ml of sodium metaperiodate reagent was added to the tubes followed by the addition of 0.5 ml of acetyl acetone reagent. The tubes were cooled and the colour developed was read at 405 nm in UV spectrophotometer against the blank. The values are expressed as mg/dl.

**Fractional Precipitation of Lipoproteins**

Lipoproteins were fractionated by dual precipitation technique as described by Wilson and Spiger, 1973.
Separation of HDL-c fractionation

Total HDL was separated by the method of Warnick and Albers, 1978.

Reagents

- Heparin-manganese chloride reagent

  3.167 g of manganese chloride was added to 1 ml of heparin containing 20,000 units/ml. This was made up to 8 ml with water.

Procedure

To 1 ml of serum 0.09 ml of heparin-manganease chloride reagent was added and mixed well. The solution was allowed to stand at 40°C for 30 minutes and then centrifuged at 2500 rpm for 30 minutes. The supernatant represented HDL fraction. Aliquot was taken from HDL fraction for the estimation of cholesterol. The level of HDL fraction was expressed as mg/dl.

Separation of LDL and VLDL

Reagents

Sodium dodecyl sulphate (SDS): 10% SDS in 150mM sodium chloride (pH 9.0).

Procedure

To 1 ml of serum 0.15 ml of sodium dodecyl sulphate was added. The contents were mixed well and incubated at 37°C for 2 hours. The contents were centrifuged in the refrigerated centrifuge at 10,000 rpm for 15 minutes. VLDL aggregated as pellet. The supernatant contained HDL and LDL fractions. Cholesterol was estimated from this fraction. The levels of LDL and VLDL fractions were expressed as mg/dl.

Atherogenic index (AI)

Atherogenic index was calculated using the formula,

\[
\text{Atherogenic index (AI)} = \frac{\text{Serum cholesterol}}{\text{Serum HDL-Cholesterol}}
\]

Estimation of free fatty acids

Free fatty acids were measured by the method of Hron and Mehahen, 1981 with the colour reagent of Itaya, 1977.

Reagents

- Activated silicic acid
Chloroform-heptane-methanol mixture (CHM): It was prepared by mixing chloroform, heptane and methanol in the ratio of 200:150:7(v/v).

Cu-TEA reagent: 50 ml of 100 mM copper nitrate and 50 ml of triethanolamin (200mM) was mixed with 33g of sodium chloride.

Colour reagent: 0.1% diethyl dithio carbonate in butanol.

Standard:
  - 2mg/ml of palmitic acid was prepared and kept as stock. For preparing a working standard this was diluted 1:10 in CHM mixture to give a concentration of 200µg/ml.

Procedure

0.1 ml of serum was added to 5.9 ml of CHM solvent and 200 mg of activated silicic acid. The Contents were shaken well and centrifuged. Standard solution in the range of 25-100 µg were also pipetted out and made up to 6 ml with CHM. The blank comprised of 6 ml CHM only. To all these samples 2 ml of Cu-TEA reagent was added and mixed well. The tubes were centrifuged to separate the 2 phases and 2 ml of the upper phase from each tube was transferred to another set of tubes. To all these tubes 1 ml of colouring reagent was added shaken well. The colour intensity was measured at 430nm in an UV spectrophotometer. The level of free fatty acids present in the serum was expressed as mg/dl.

Estimation of phospholipids

Phospholipids present in the serum sample was estimated by the method of Bartlett, 1959.

Perchloric acid.

Ammonium molybdate reagent: 2.5 % ammonium molybdate in 5 N sulphuric acid.

Aminonaphthol sulphonic acid (ANSA): Dissolve 500 mg ANSA in a mixture of 195 ml of 15% sodium bisulphate and 5 ml of 20 % sodium sulphite solution.

Standard phosphorous solution: Dissolve 35.1 mg of potassium dihydrogen phosphate in 100 ml of distilled water. Prepare the working standard by diluting this stock solution in the ratio of 1:10 to give a concentration of 80 µg phosphorous/ml.
**Procedure**

The standard solution was pipetted out from 0.2-1.0 ml into a series of test tubes labelled as S1-S5. 1.0 ml of serum sample was taken in the test tube marked as T, and then add 0.5 ml of perchloric acid to all the tubes. This mixture was kept in a sand bath until they become colourless. Then all the tubes were made up to 4.3 ml with distilled water. Then add 0.5 ml of ammonium molybdate solution. Then tubes were incubated at room temperature for 10 minutes followed by the addition of 0.2 ml of ANSA and incubated at room temperature for 20 minutes. The colour developed was measured at 660nm against blank sample. The standard graph was drawn to measure the amount of phospholipid present in the sample. The amount of phospholipids present in the serum were expressed as mg/dl.

**Estimation of Glucose**

Blood glucose was estimated by the method of *Sasaki et al., 1972*.

**Reagents**

- 10 % TCA
- Ortho- Toluidene Reagent
- 12.5 g of thiourea and 12 g of boric acid were dissolved in 50 ml of distilled water by heating over a mild flame. 75 ml of redistilled O-Toluidene and 375 ml of acetic acid were mixed with thiourea boric acid mixture and the total volume was made up to 500 ml with distilled water. The reagents was left in a refrigerator and filtered.
- Standard Glucose Solution
  
  10mg of pure glucose was dissolved in 100ml of 0.2% benzoic acid in water.

**Procedure**

0.1 ml of blood was mixed with 1.9 ml of TCA solution to precipitate proteins and then centrifuged. 1.0 ml of the supernatant was mixed with 4ml of O-Toluidene reagent and kept in boiling water for 15 minutes. The colour developed was read at 600nm in a colorimeter. A set of standard glucose were also treated similarly. The values were expressed as mg/dl.
Determination of Insulin

Insulin levels were assayed using a standard mercodia rat Insulin ELISA enzyme assay kit from Mercodia, Sweden(Cat. No-10-1124-01). The assay was performed according to manufacturer’s protocol. The values were expressed as µU/ml.

Homeostatic Model Assessment- Insulin Resistance (HOMA-IR)

The homeostatic model assessment (HOMA) is a method used to quantify insulin resistance. It was measured by using the formula,

\[ \text{HOMA-IR} = \frac{\text{Glucose (mg/dl)} \times \text{Insulin (µU/ml)}}{405} \]

Estimation of Total Protein

The total proteins were determined using Folin – Ciocalteau reagent (Lowry et al., 1951). The phenolic group of tyrosine and tryptophan residues in protein produce a blue purple colour complex, with maximum absorption in the region of 660 nm wavelength, with Folin-Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of colour developed depends on the amount of these aromatic amino acids present and thus vary for different proteins.

Reagents

★ BSA stock solution (1mg/ml)

★ Analytical Reagents:
  o Solution A
    o 50 ml of 2% sodium carbonate was mixed with 50 ml of 0.1 N NaOH solutions. (0.4 gm in 100 ml distilled water)
  o Solution B
    o 10 ml of 1.56% copper sulphate solution was mixed with 10 ml of 2.37% sodium potassium tartarate solution.
    o Prepare analytical reagents by making 2 ml of (b) with 100 ml of (a)

★ Folin-Ciocalteau reagent solution-Dilute commercial reagent with 1:2 volume of distilled water on the day of use.

Procedure

0.2 ml of serum was taken in the test tube and 2ml of alkaline copper reagent were added and mixed well. Bovine serum albumin solution and water acts as standard and blank respectively to which 2 ml of alkaline copper reagent was added. All the tubes were incubated at room temperature for 10 minutes. 0.2ml of Folin-Ciocalteau reagent solution was added to all the test tubes and incubated for 30 minutes. The colour
developed was read at 660 nm using spectrophotometer. The values were expressed as g/dl of serum.

**Estimation of Serum Albumin**

Serum albumin was estimated by dye binding method (Doumas *et al.*, 1971). Albumin binds specifically to bromocresol green (BCG). The intensity of green colour formed by albumin BCG complex is directly proportional to the albumin concentration in serum which is read at 630 nm.

**Reagents**

**Standard albumin solution**

- 200 mg of bovine serum albumin was dissolved in 100 ml of distilled water and 2 ml of standard was diluted with 100 ml of distilled water which acts as working standard. (40 µg/ml)

**Procedure**

0.1 ml of serum is mixed with 3 ml of albumin colour reagent (BCG). 0.1 ml of sodium chloride was treated as the blank. 3 ml of albumin colour reagent was added to the test tubes containing blank and standard solution. All the tubes were mixed well and allowed to stand for 10 minutes. The colour formed was read at 630 nm. The values were expressed as g/dl of serum.

**Estimation of Globulin**

The globulin level in serum was calculated using the formula given below,

\[
\text{Globulin} = \text{Total protein} - \text{Albumin}
\]

**Assay of Serum Glutamate Oxaloacetate Transaminase (AST) [E C 2.6.1.1]**

The activity of SGOT was analysed by the method of King, 1965(a).

**Reagents**

- Phosphate buffer: 100mM, pH:7.4
- Substrate: 2.66 gms of DL- Aspartate and 32 mg of L – α ketoglutarate were dissolved in 20.5 ml of 1N Sodium hydroxide by gentle heating. This is made up to 100 ml with buffer.
- Dinitrophenyl hydrazine : 1mM of Dinitrophenyl hydrazine in 2N Hydrochloric acid.
- Sodium hydroxide: 0.4N solution.
- Standard pyruvate: 11mg of Sodium pyruvate was dissolved in 100ml of phosphate buffer.
**Procedure**

In different tubes, 1 ml of buffered substrate was added. 0.1 ml of serum/homogenate was added and incubated at 37°C for one hour. Then 1.0 ml of Dinitrophenyl hydrazine reagent was added to arrest the reaction. To the blank, 0.1 ml of serum/homogenate was added after the addition of Dinitrophenyl hydrazine reagent. The tubes were kept aside for 15 minutes, and then 10ml of sodium hydroxide was added and read at 520nm in a UV spectrophotometer. The enzyme activity was expressed as IU/L.

**Assay of Serum Glutamate Pyruvate Transaminase (ALT) [EC 2.6.1.2]**

The activity of SGPT was assayed by the method of King, 1965 (b).

**Reagents**

- Phosphate buffer: 100mM, pH 7.4
- Substrate: 1.78gm L- Alanine and 38 mg of α ketoglutarate were dissolved in buffer. 0.5 ml of 1N Sodium hydroxide was added by gentle heating. This is made upto 100 ml with buffer.
- Dinitrophenyl hydrazine: 1mM dinitrophenyl hydrazine in 2N hydrochloric acid
- Sodium hydroxide: 0.4N Solution
- Standard pyruvate: 11mg of sodium pyruvate was dissolved in 100ml of phosphate buffer.

**Procedure**

In different tubes, 1.0 ml of buffered substrate was added. 0.1ml of serum was added and incubated at 37°C for 30 minutes. Then 1.0 ml of dinitrophenyl hydrazine reagent was added to arrest the reaction. To the blank, 0.1 ml of serum was added after the addition of Dinitrophenyl hydrazine reagent. The tubes were kept aside for 15 minutes and then 10 ml of Sodium hydroxide was added and read at 520nm in a UV Spectrophotometer. The enzyme activity was expressed as IU/L.

**Assay of Alkaline Phosphatase (ALP) [EC 3.1.3.1]**

ALP was assayed by the method of King, 1965(a).

**Reagents**

- Sodium carbonate – sodium bicarbonate buffer, pH 10.
- Disodium phenyl phosphate
- 0.5N Sodium Hydroxide
- 20% Sodium carbonate
Folin phenol’s Reagent

Procedure

In different tubes, 1.0 ml of buffered substrate was added and preincubated for 37°C for 5 minutes followed by 0.1 ml of serum/homogenate and incubated at 37°C for 15 minutes. Then 0.8 ml of NaOH was added to arrest the reaction. To the blank, 0.1 ml of serum was added after the addition of NaOH. The contents were centrifuged for 2 minutes. 1.0 ml of supernatant was taken and made up to 4 ml with distilled water. Blank comprises of 4 ml of distilled water. 0.5 ml of Folin’s Phenol reagent and 1.5 ml of Na₂CO₃ were added to all the tubes including the blank and read at 640 nm in UV spectrophotometer. The enzyme activity was expressed as IU/L.

Estimation of Lactate dehydrogenase (LDH) [EC 1.1.1.27]

The activity of lactate dehydrogenase (LDH) was measured by the method of King, 1965.

Reagents

- Glycine buffer (0.1M), 7.5 g of glycine and 5.85 g of sodium chloride were dissolved in 1 litre of distilled water.
- Buffered substrate: 2.78 g of lithium lactate was dissolved in 14 ml of glycine buffer containing 75 ml of 0.1 N sodium hydroxide solution. This was prepared just before use.
- NAD+: 20 mM
- DNPH: 0.2% of DNPH in 1 N hydrochloric acid.
- Sodium hydroxide: 0.4 N
- Standard pyruvate solution: 11 mg of sodium pyruvate was dissolved in 100 ml of buffer.

Procedure

To a set of tubes, 1.0 ml of the buffered substrate and 0.1 ml of serum was added and the tubes were incubated at 37°C for 15 minutes. After adding 0.2 ml of NAD solution, the incubation was continued for another 15 minutes. The reaction was then arrested by adding 1.0 ml of DNPH reagent and the tubes were incubated for further period of 15 minutes at 37°C. 0.1 ml of serum was added to the blank tubes after arresting the reaction with DNPH. 7 ml of sodium hydroxide solution was added and the colour developed was measured at 420 nm in a UV spectrophotometer. Suitable aliquotes of standard were also analysed by the same procedure.
Determination of creatinine phosphokinase (CPK) [EC 2.7.3.2]

The creatinine phosphokinase activity was measured by using Diagnostic kit of Agappe diagnostic Ltd, India. The assay was performed according to manufacturer’s protocol.

The activity of enzyme was expressed as IU/L.

Determination of Lipase activity [EC 1.3.3.1]

Lipase activity in serum was measured by the kit method (Agappe diagnostic Ltd, India). The assay was performed according to manufacturer’s protocol.

The activity of enzyme was expressed as IU/L.

Determination of Homocysteine

Homocysteine present in plasma sample was measured by the commercially available kit of Primus et al., 1988.

Determination of Leptin and adiponectin

Serum leptin (Mizuno et al., 2003) and adiponectin (Meada et al., 1996) levels were measured by ELISA using commercially available kits.

Determination of Apolipoprotein -B (Apo-B)

Apo lipoprotein B present in the sample was measured using the standard kit by the method of Wieland et al., 1982.

Collection of Tissue Samples

Rats were treated as per the treatment protocol. After the experimental period the animals were sacrificed by cervical decapitation. Tissue specimens such as liver, kidney, spleen, pancreas, heart, brain and adipose tissue were collected, sample was excised immediately and kept in physiological saline and weight of the organs were noted. Liver and pancreas homogenate was prepared in 0.01M Tris-HCL buffer (pH 7.4) Tissue homogenates were used for further analysis.

Liver and serum was used for enzymatic and non-enzymatic antioxidant assays. Pancreas was used for the assay of pancreatic lipase activity.

Pancreatic lipase assay

Pancreatic lipase activity was measured by the method of Kanwar et al., 2005.

Reagents

★ 0.05 M tris buffer pH 8.5
★ p-nitro phenol
**Procedure**

The pancreas from each animal was removed and the pancreatic lipase was assayed. A stock solution of p-nitro phenol was prepared in 0.05M Tris buffer at pH 8.5. Different dilutions of p-nitro phenol was prepared and used as the standard. Pancreatic lipase (100mg of tissue used to prepare tissue homogenate) acts on p-nitro phenyl palmitate (pNPP) to release yellow coloured p-nitro phenol which was measured spectrophotometrically. The reaction was carried out at 45ºC for 20 minutes. The release of p-nitro phenol was measured spectrophotometrically at 410nm. The blank was run with the same reaction mixture heated in boiling water bath for 10 minutes. Pancreatic lipase activity was expressed as units/mg of protein.

**Assay of Lipid peroxides (LPO)**
The level of lipid peroxides was assayed by the method of Ohkawa et al., 1979.

**Reagents**
- 8.1% of Sodium dodecyl sulphate (SDS)
- 20% Acetic acid, pH 3.5 adjusted with NaOH
- 0.8% Thiobarbituric acid (TBA)
- n- Butanol/ pyridine mixture (15:1 v/v)
- 1,1,3,3- tetra methoxypropane

**Procedure**
To 0.2 ml of homogenate, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4ml with water and then heated in an oil bath at 90 °C for 60 minutes. After cooling, 1.0 ml of water and 5 ml of n-butanol/ pyridine mixture were added. After centrifugation at 4000 rpm for 10 minutes, the absorbance of organic layer was measured at 532 nm. The level of lipid peroxides was expressed as nmoles/ml.

**ENZYMATIC ANTIOXIDANTS**

**Assay of Superoxide Dismutase (SOD) [EC 1.15.1.1]**
The activity of superoxide dismutase was determined by the method of Marklund and Marklund 1974.

**Reagents**
- 0.1M Tris-HCl buffer, pH 8.2 containing 2mM of diethylene triamine penta acetic acid.
- 0.05M Tris – HCl buffer, pH 7.4.
- **Pyrogallol solution**: 25.2 mg was dissolved in 1.0 ml of 0.05 M Tris-HCl buffer (pH 7.4) in an aluminium foil wrapped and stoppered test tube.

- **Pyrogallol working solution**: At the time of assay, 0.5 ml was diluted to 50 ml with the 0.05 M Tris-HCl buffer, pH 7.4.

- **Absolute ethanol**.

- **Chloroform**

**Procedure**

To a known amount of tissue aliquot or serum, 0.25 ml of ethanol and 0.15 ml of chloroform were added. After 15 minutes of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant obtained constituted the enzyme extract. The reaction mixture for auto-oxidation consisted of 2ml of Tris-HCl buffer (pH 8.2), 0.5 ml of 2 mM pyrogallol and 1.5ml of water. Initially the rate of auto-oxidation of pyrogallol was noted at an interval of 1 minute to 3 minutes. The assay mixture for the enzyme contained 2ml of the buffer, 0.5 ml of pyrogallol, aliquots of the enzyme preparation and water to give a final volume of 4ml. The rate of inhibition of pyrogallol auto-oxidation after the addition of the enzyme was noted. The enzyme activity was defined as the enzyme required for 50% inhibition of pyrogallol auto-oxidation (units/mg protein).

**Assay of catalase [EC 1.11.1.6]**

The activity of catalase was assayed by the method of Sinha, 1972.

**Reagents**

- **0.01M phosphate buffer, pH 7.0**
- **0.2M H₂O₂**
- **Stock dichromate / acetic acid solution**: This reagent was prepared by mixing 5% solution of potassium dichromate with glacial acetic acid (1:3 by volumes)
- **Working dichromate/acetic acid solution**: The stock was diluted to 1:5 with water to prepare the working dichromate/acetic acid solution.

**Procedure**

The assay mixture contained 0.5 ml of Hydrogen peroxide (H₂O₂), 1ml buffer and 0.4ml water. 0.2ml of the diluted enzyme was added to initiate the reaction. 2ml of the dichromate/acetic acid reagent was added after 15, 30, 45 and 60 seconds of incubation. To the control tube, the enzyme was added after the addition of the acid reagent. The
tubes were then heated for 10 minutes and colour developed was read at 610 nm. The activity of catalase is expressed as μ moles of H₂O₂ consumed/min/mg protein.

**Assay of glutathione peroxidase (GPx) [EC 1.11.1.9]**
The activity of GPx was assayed by the method of Rotruck et al., 1973.

**Reagents**

- 0.32 M Phosphate buffer, pH 7.0
- 0.8mM EDTA
- 10mM Sodium azide
- 3mM reduced glutathione
- 2.5mM H₂O₂
- 10% TCA
- 0.3M Disodium hydrogen phosphate
- DTNB solution (40 mg of DTNB in 100 ml of 1% sodium citrate)
- Reduced glutathione

**Procedure**
The reaction mixture consisted of 0.2 ml each of EDTA, sodium azide, H₂O₂, 0.4 ml of phosphate buffer, 0.1 ml homogenate/serum and was incubated at 37°C and reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged at 2000 rpm. 0.5 ml of supernatant, 4 ml of disodium hydrogen phosphate and 0.5ml DTNB were added and the colour developed was read at 420 nm immediately.

The activity of GPx was expressed as μ moles of glutathione oxidized/min/mg protein.

**NON–ENZYMATIC ANTIOXIDANTS**

**Estimation of Reduced Glutathione (GSH)**
The concentration of reduced glutathione was measured by the method of Moron et al., 1979.

**Reagents**

- 10% TCA
- 0.6 mM 5, 5'-dithiobis-2-nitrobensoic acid (DTNB) in 0.2M sodium phosphate
- 0.2M Phosphate buffer, pH 8.0

**Procedure**
1 ml of homogenate/serum was precipitated with 1 ml of TCA and the precipitate was removed by centrifugation. To 0.5 ml of supernatant, 2 ml of DTNB was added and
the total volume was made up to 3ml with phosphate buffer. The absorbance was read at 412 nm.

The level of glutathione was expressed as µg/mg protein.

**Estimation of Ascorbic Acid**

The concentration of ascorbic acid was estimated by the method of Omaye *et al.*, 1979.

**Reagents**

- 5% TCA
- 65% H$_2$SO$_4$
- DNPH-Thiourea-CuSO$_4$ (DTC) reagent: 3g DNPH, 0.4g Thiourea and 0.05g CuSO$_4$ was dissolved in 9N H$_2$SO$_4$ and made up to 100 ml with the same.
- Standard: Ascorbic acid was made with 5% TCA in the range of 0 to 20 mg/ml.

**Procedure**

Aliquots of homogenate or serum precipitated with 5% ice cold TCA and centrifuged for 20 minutes at 3500g. 1ml of the supernatant was mixed with 0.2ml of DTC and incubated for 3 hours at 37°C. Then 1.5 ml of ice cold 65% H$_2$SO$_4$ was added, mixed well and the solution was allowed to stand at room temperature for an additional 30 minutes. Absorbance was determined at 520 nm. The concentration of ascorbic acid in the tissue was expressed as mg/mg protein. The concentration of ascorbic acid in serum was expressed as mg/dl.

**Estimation of Vitamin E**

The level of vitamin E was estimated by the method of Desai, 1984.

**Reagents**

- Ethanol
- Petroleum ether
- 0.2% bathophenanthroline in ethanol
- 0.001 M Ferric chloride in ethanol
- 0.001 M Ortho-phosphoric acid in ethanol
- α-Tocopherol acetate

**Procedure**

To 1.0 ml of homogenate or serum, 1ml of ethanol was added and thoroughly mixed. Then 3 ml of petroleum ether was added, shaken rapidly and centrifuged. 2 ml of supernatant was taken and evaporated to dryness. To this 0.2 ml of bathophenanthroline
was added. The assay mixture was protected from light and 0.2 ml of ferric chloride was added followed by 0.2 ml of Ortho-phosphoric acid. The total volume was made up to 3ml with ethanol. The colour developed was read at 530 nm. The concentration of vitamin E was expressed as mg/mg protein. The concentration of vitamin E in serum was expressed as mg/dl.

**Statistical analysis**

The data were expressed as mean ± SD. All statistical analysis was performed using SPSS 20.0 statistical software (IBM, USA). Significant differences among the treatment groups were analysed by variance (One way ANOVA) followed by least significant difference (LSD) test. Results were considered to be statistically significant at P values < 0.05. Graphs for this study were plotted using graph pad prism version 6.02.

**Histopathological Examination**

Tissue specimens like liver, kidney, spleen pancreas, heart and adipose tissue were fixed in 10% buffered formalin for atleast 4 hours immediately after sacrifice. Specimens were dehydrated by passing through ascending grades of alcohol, cleared in xylene, impregnated and embedded in paraffin. These sections were cut (3–5 µm) and were stained using haematoxylin eosin and mounted in DPX and visualized under microscope for histological changes.

**Oil red O staining**

Lipid content present in the liver tissue was measured using oil red O staining by the method of **Preece, 1972.**

**Reagents**

- Oil Red O 0.7%
- Propylene glycol
- 85% propylene glycol

**Procedure**

- Cut fresh frozen liver tissue sections at 5-10 µm thick and mount on slides.
- Then slides were air dried for 30-60 minutes at room temperature and then fixed in ice cold 10% formalin for 5-10 minutes. And then rinse the slide with water and air dry for 30-60 minutes.
- Then add Propylene glycol for 5 minutes.
- After that add Oil red O stain for 7 minutes and agitate the slides.
- Then add 85% Propylene glycol for 3 minutes.
- Then slides were rinsed in distilled water.
- Then slides were mounted with aqueous mounting media, Glycerin Jelly.
- Stained liver was visualized under microscope to determine histological changes.

**Results and discussion**

Obesity is a global health problem, resulting from an energy imbalance caused by increased ratio of caloric intake to energy expenditure. Genetic predisposition, changes in life style and diet are the causes for the obesity and obesity related consequences such as cancer, aging, cardiovascular diseases and number of pathological conditions including type II diabetes. Obesity is regarded as disorder of lipid metabolism and enzymes involved in this process could be useful to develop antiobesity drugs. Antiobesity drug such as orlistat reduces intestinal fat absorption by inhibiting pancreatic lipase. While using sibutramine, it inhibits the uptake of serotonin and norepinephrine. Due to the disadvanges of commercially available drugs, there is a need for new drug from the natural products that are used as antiobesity agent to treat obesity.

**Incidence of Obesity in experimental animals**

The incidence of obesity was noted after the experimental periods of high fat diet fed rats and was confirmed by the accumulation of visceral fat, body mass index and body weight. The experimental animals were fed with high fat diet and simultaneously treated with EEBH (300mg/kg body weight), AFBH (100mg/kg body weight) and orlistat (standard drug 25mg/kg body weight). Group II or high fat diet fed animals (obesity animals) showed high visceral body mass. This was compared to the normal animals (Group I) and EEBH treated (Group III) and Group IV (AFBH treated) and standard drug (Group V) animals. The physical appearance of the animals was shown in **Figure 5.5**. Group II rats showed significant increase in the body weight as compared with Group I animals. Group III, Group IV and GroupV animals showed significant decrease in the body weight.

**Body weight changes**

Physical parameter such as body weight changes, Thoracic circumference (TC) and Abdominal circumference (AC) changes was observed during the experimental period.

Body weight of an individual is increased to a great extent. Reducing body weight and body fat are important parameters in preventing obesity (**Flegal et al., 2002**).
Excess of rich food intake and lack of physical exercise leads to the accumulation of body fat and adipose tissue stores excess of energy in the form of lipids, free fatty acids is liberated from lipoproteins by the activity of lipoprotein lipase and enter into the adipocyte where it is reassembled as triglycerides.

All the experimental animal groups were healthy and did not show any abnormalities during the experimental period. **Figure 5.6 and Table 5.1** showed the body weight changes during the experimental period and weight gain in the obesity induced Group II is about 356 ± 2.64 and weight reduction was shown in group III(HFD+EEBH treated) is about 325 ± 3.00 and Group IV has 303.33 ± 2.08 (HFD+AFBH treated). After the 8 weeks of experimental period Group II or HFD fed rats showed significant (P<0.001) increase in body weight when compared with that of normal control. Comparing EEBH treated HFD animals with AFBH treated animals

**Figure 5.5: Obesity incidence in experimental animals**

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Control animals,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>- HFD fed animals (Obesity induced animals),</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>- Obesity induced animals treated with EEBH (300mg/kg body wt),</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>- Obesity induced animals treated with AFBH (100mg/kg body wt),</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>- Obesity induced animals treated with orlistat (25mg/kg body wt).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.6: Body weight changes for 8 weeks in experimental animals

Each Value is expressed as mean± SD for six animals in each group

Group I- Control animals,
Group II- HFD fed animals (Obesity induced animals),
Group III- Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV- Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V- Obesity induced animals treated with orlistat (25mg/kg body wt).

showed better weight reduction after the experimental period. Group I and Group II animals were compared with Group III, Group IV and Group V animals.

EEBH and AFBH treated rats do not exert any side effects during the experimental periods. This shows the extract and active fraction does not contain toxic
substances. No death was observed during the experimental period. No abnormal changes were observed in experimental animal groups.

Mopuri and Meriga in 2014 explained about the body weight changes in high calorific diet fed rats. High calorific diet fed rats consumes more feed than high calorific diet treated with Terminalia paniculata bark. The Terminalia paniculata bark reduces the body weight.

Lee et al., 2011 stated the changes in body weight in high fat diet fed rats. Obx mice regulate obesity with low fat diet and a high fat diet. Body weight is significantly increased (P<0.01) in high fat diet when it was compared with low fat diet treated experimental animals.

Barakat and Mahmoud, 2011 demonstrated that body weight was increased in high fat diet fed rats. The body weight of animals on the high fat diet showed rapid increase during the experimental period of 8 weeks. High fat diet fed animals were treated with purslane extract showed a gradual decrease in body weight gain.

Hemalata et al., 2013 explained the body weight changes in high fat diet fed rats. High fat diet group showed significant increase in body weight as compared to control group. The administration of stigmasterol and sibutramine prevented the body weight gain as compared to high fat diet fed animals.

Yamamoto and Oue in 2006 reported the body weight gain in high fat sucrose diet. Body weight gain is significantly decreased in quercetin treated rats when compared with normal and high fat diet fed rats.

Malik and Sharma et al., 2011 proved that the body weight of high fat diet fed animals was significantly increased. The body weight is significantly decreased in high fat diet fed rats treated with ginger extract.

Pushpalatha et al., 2011 proved that high fat diet fed rats showed significant increase of body weight when compared with high fat diet fed rats treated with Achyranthus aspera. Achyranthus aspera treated animals showed significant decrease in the body weight.

Feed intake

The feed consumption of different groups was shown in Table 5.1. There was a significant (P<0.001) increase in feed consumption of Group II animals when compared with that of normal Group I animals. The AFBH treated Group IV animals showed significantly (P<0.001) decrease in feed consumption during the experimental period.
Orlistat treated Group V animals were found to show significant (P<0.001) decreases in feed consumption during 8 weeks period. As shown in Table 5.1, the body weight gain of the group II animals was actually due to the increased feed intake. However, the body weight of the AFBH and EEBH treated on high fat diet fed rats was significantly reduced, despite a decrease in food consumption compared to the Group II animals. The decreased food consumption was showed in the AFBH treated animals when compared with that of EEBH treated high fat diet animals. AFBH treated animals showed best result than EEBH treated animals.

Barakat in 2011 reported that food intake was increased in HFD fed animals. High fat diet treated with purslane extract showed a gradual decrease in the food intake during the experimental period.

Yamamoto and Oue in 2006 proved that high fat diet rats consumes large amount of food when compared with the high fat diet fed rats treated with quercetin. Quercetin treated animals showed less quantity of food consumption.

The food intake in obese animals was significantly increased during 8 weeks and this was proved by Retnasamy and Adikay, 2014.

Anthropometrical Determinations.

Rodrigues et al in 2012 explained about the positive correlation between daily lipid intake and BMI as well as fat deposition.

Thoracic Circumference (TC) and Abdominal Circumference (AC) were significantly increased in HFD fed animals. BMI also showed significant increase in obese rats when compared with control animals. These results indicated that there was an increase in fat accumulation in the region of thoracic and abdomen.
Table 5.1: Effect of AFBH and EEBH on Body weight and Food intake in experimental animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight(g)</td>
<td>200.0 ± 1.00</td>
<td>200.33 ± 2.08</td>
<td>202.33 ± 1.52</td>
<td>206.34 ± 6.80</td>
<td>202.00 ± 2.64</td>
</tr>
<tr>
<td>Final body weight(g)</td>
<td>288.30 ± 7.37</td>
<td>356.00 ± 2.64</td>
<td>325 ± 3.00 a b*</td>
<td>303.33 ± 2.08 a b*</td>
<td>316 ± 1.00 a b*</td>
</tr>
<tr>
<td>Weight gain(g)</td>
<td>88.33 ± 6.65</td>
<td>155.66 ± 3.51 a*</td>
<td>122.66 ± 2.88 a b*</td>
<td>97.00 ± 8.88 aNS b*</td>
<td>114 ± 3.60 a b*</td>
</tr>
<tr>
<td>Food intake(g/day)</td>
<td>17.40 ± 0.10</td>
<td>19.53 ± 0.11 a*</td>
<td>19.20 ± 0.10 a b*</td>
<td>17.46 ± 0.28 a b*</td>
<td>18.50 ± 0.17 a b*</td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group.

Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.
Comparison: a-as Compared with Group I; b-as Compared with Group II.

The anthropometrical determinations of different groups were showed in Table 5.1a. Thoracic circumference is significantly increased (P<0.001) in Group II animals when compared with Group I animals. However, significant (P<0.001) decrease in thoracic circumference of animals treated with AFBH (Group IV) was shown. The thoracic circumference of orlistat (Group V) animals showed significant decrease (P<0.001) when compared with the Group II animals.

The abdominal circumference was significantly increased (P<0.001) in group II animals when compared with normal (Group I) animals. Group III, Group IV and Group V animals showed significant decrease (P<0.001) when compared with group II animals and Group I. The changes in abdominal circumference are shown in Table 5.1a.
The Body mass index (BMI) showed a significant increase (P<0.001) in Group II animals when compared with normal animals (Group I). Group III, Group IV and Group V animals showed significant decrease (P<0.001) when compared with Group II animals and Group I animals. Table 5.1a shows the result of BMI changes. BMI also decreased in Group IV (AFBH) treated animals and revealed best result than EEBH and Group V (orlistat) treated animals.

Shahat et al., 2012 explained about the anthropometric parameters such as Thoracic Circumference (TC) and Abdominal Circumference (AC) were significantly increased in obese rats when compared with the plantoga seed treated and fennel extracts treated with high fat diet fed rats. BMI also increased in obese rats. Extract treated animals showed decrease in the levels of BMI.

Novelli et al., 2007 showed that the intake of high fat diet increased in high fat diet fed rats, which in turns increases the body weight due to excessive energy intake and was deposited in the adipose tissue. Body mass index is a simple and reliable parameter to estimate the body fat in obesity rats.

Deshpande et al., 2013 denoted that anthropometric parameters such as Thoracic circumference(TC) and Abdominal circumference(AC) was significantly increased in obese rats which were compared with Ziziphus mauritiana treated rats. These results were similar to our results.

Similar results were obtained in high fat diet treated with EEBH and AFBH treated rats.

Table 5.1a: Effect of AFBH and EEBH on Anthropometrical determination in experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracic circumference (TC in cm)</td>
<td>13.20 ± 0.10</td>
<td>14.20 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.80 ± 0.10&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;*,b&lt;/sup&gt;</td>
<td>13.4 ± 0.57&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;*,d&lt;/sup&gt;</td>
<td>13.6 ± 0.57&lt;sup&gt;a,t&lt;/sup&gt;&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;*,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abdominal circumference (AC in cm)</td>
<td>6.9 ± 0.10</td>
<td>9.10 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.85 ± 0.05&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;*,b&lt;/sup&gt;</td>
<td>7.10 ± 0.10&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;<em>,d&lt;/sup&gt;&lt;sup&gt;</em>,e&lt;/sup&gt;</td>
<td>7.50 ± 0.10&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;<em>,b&lt;/sup&gt;&lt;sup&gt;</em>,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI</td>
<td>0.67 ± 0.01</td>
<td>1.00 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82 ± 0.06&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;*,b&lt;/sup&gt;</td>
<td>0.74 ± 0.02&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;<em>,d&lt;/sup&gt;&lt;sup&gt;</em>,e&lt;/sup&gt;</td>
<td>0.77 ± 0.01&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;B&lt;/sup&gt;&lt;sup&gt;<em>,d&lt;/sup&gt;&lt;sup&gt;</em>,e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Each Value is expressed as mean ± SD for six animals in each group:

- Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
- Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
- Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
- Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.

Organ weight

Animals fed with high fat diet showed increase in weight of the internal organs like heart, liver, spleen, kidney, pancreas and intestine when compared with normal control animal (Ganachari et al., 2007).

Organ weight such as heart, liver, spleen, kidney, pancreas and brain weight was noted. Organ weight of heart, liver, spleen, kidney and pancreas of Group II animals were compared with Group I normal control (Table 5.2). Group II animals showed significantly increased organ weight (P<0.001) than high fat diet treated AFBH Group IV and Group III animals showed significantly decreased weight of organs P<0.001 and also standard Orlistat treated animals (Group V). These results are compared with group I and group II animals. The organ weight changes are shown in Table 5.2.

AFBH treated animals showed better result than other groups. No significant changes were observed in the organ weight of brain.

Kameshwaran et al., 2013 explained about changes in the organ weight and reported that increase in atherogenic diet induce obesity in rats. Methanolic extract of Tecoma stans flowers treated animals showed decrease in the organ weight.

Supriya et al., 2012 showed the changes in the organ weight like liver, kidney, spleen, kidney and adipose tissue. They stated that the weight of such organs was increased in high fat diet fed rats. The changes in the Shorea robusta G. leaf extract treated high fat diet showed a decreased level in organ weight.
Norazmir and Ayub, 2010 showed that there was a change in the organ weight like liver, kidney, spleen and kidney in high fat diet induced obese animals. The Pink guava puree treated high fat diet induced obese rats showed a decrease in the organ weight.

Organ weight changes in high fat diet fed rats showed increase in the weight of the organs such as liver, kidney and spleen. This organ weight is decreased in Cyperus rotandus treated animals (Athesh et al., 2014).

Fat pad weight

Fat pad weight such as perirenal, mesenteric, epididymal and gonadal fat pad weight was assessed. This is shown in Table 5.2a.

| Table 5.2: Effect of AFBH and EEBH on Organ weight in experimental animals |
|--------------------------|--------|--------|--------|--------|--------|
| Parameter | Group I | Group II | Group III | Group IV | Group V |
| Pancreas(g) | 1.32 ± 0.08 | 1.61 ± 0.02 a* | 1.24 ± 0.01 a#b* | 0.92 ± 0.02 a*b* | 0.90 ± 0.06 a*b* |
| Liver(g) | 7.45 ± 0.45 | 11.91 ± 1.08 a* | 9.34 ± 0.34 a*b* | 8.41 ± 0.11 a#b* | 8.75 ± 0.05 a*b* |
| Kidney(g) | 1.68 ± 0.02 | 2.24 ± 0.11 a* | 2.15 ± 0.04 a*b@ | 1.92 ± 0.07 a*b* | 1.83 ± 0.02 a*b* |
| Spleen(g) | 0.81 ± 0.03 | 1.27 ± 0.09 a# | 0.98 ± 0.02 a*b* | 0.87 ± 0.04 a@b* | 0.84 ± 0.03 aNSb* |
| Heart(g) | 0.83 ± 0.01 | 1.16 ± 0.07 a* | 1.04 ± 0.01 a*b* | 0.93 ± 0.08 a#b* | 1.01 ± 0.04 a*b* |
| Brain(g) | 1.51 ± 0.01 | 1.55 ± 0.05 a@ | 1.53 ± 0.03 aNSbNS | 1.52 ± 0.06 aNSbNS | 1.54 ± 0.02 aNSbNS |

Each Value is expressed as mean ± SD for six animals in each group

Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.
Comparison: a-as Compared with Group I; b-as Compared with Group II.
Table 5.2a: Effect of AFBH and EEBH on Fat pad weight in experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perirenal(g)</td>
<td>0.43 ± 0.02</td>
<td>2.22 ± 0.02</td>
<td>1.84 ± 0.01</td>
<td>1.52 ± 0.02</td>
<td>1.12 ± 0.02</td>
</tr>
<tr>
<td>Mesentric(g)</td>
<td>0.61 ± 0.01</td>
<td>3.14 ± 0.03</td>
<td>1.44 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>Epididymal(g)</td>
<td>0.43 ± 0.02</td>
<td>2.23 ± 0.02</td>
<td>1.51 ± 0.01</td>
<td>1.16 ± 0.02</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td>Gonadal(g)</td>
<td>1.51 ± 0.01</td>
<td>3.82 ± 0.02</td>
<td>2.32 ± 0.02</td>
<td>1.92 ± 0.03</td>
<td>0.91 ± 0.01</td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group.

- Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
- Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
- Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
- Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: *- P < 0.001.
Comparison: a-as Compared with Group I; b-as Compared with Group II.

Fat pad like perirenal, mesenteric, epididymal and gonadal fat pad weight increased in Group II high fat diet fed rats (P<0.001) when compared with Group III, Group IV and Group V animals. Group III and Group IV animals significantly decreased the weight of fat pad. When comparing Group IV (AFBH treated) and Group III (EEBH treated) animals Group IV (active fraction) treated animals showed significant decrease weight of fat pad.

Thus fat pad weight comparison was compared to other groups and from the experimental results it can be proved that AFBH showed that fat pad weight of the animals was decreased than other groups.
Fat pad weight increased in high fat diet fed rats (Group II animals). Fat pad weight like epididymal fat pad and peritoneal fat pad was increased in high fat diet fed rats. *Coccinia indica* treated with high fat diet fed animals showed significant decrease in the weight of fat pad (*Ahmed Mansoor and Manoj, 2012*).

Malik and Sharmaa in 2011 reported that changes in the fat pad weight like epididymal, retroperitoneal and mesenteric fat pad. In high fat diet fed rats the fat pad weight was increased when compared with the *Zinger officinate* treated animals. Similar result was obtained in AFBH and EEBH treated high fat diet fed animals.

*Cyperus rotundus* aqueous extract on high fat diet fed rats reduces the mesenteric and perirenal fat pad. High fat diet fed rats showed increased weight of perirenal and mesenteric fat pad. This was reported by *Athesh et al., 2014*.

**Lipid profile**

Abnormalities in the lipid metabolism are the main causes of dyslipidemia. This is a major risk of cardiovascular disease, obesity and cholesthisis. The plasma cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol via bile and feces. Excess of fat accumulation results in fatty liver condition (*Choi et al., 2001*), and it causes Fatty infiltration and chronic damage in hepatocytes. Fatty liver condition plays an important role in control of cholesterol homeostasis.

The feeding of high fat diet results in excess hepatic triglycerides accumulation due to increased synthesis and decreased secretion of triglycerides and increased de novo lipogenesis (*Liu et al., 1995*). have significantly decreased (P<0.001) level of total cholesterol, triglycerides and Low density lipoprotein (LDL). This result was compared with Group I (normal animals) and Group II animals.

The levels of HDL cholesterol significantly decreased (P<0.001) in Group II animals when compared to Group I animals. In case of EEBH treated (Group III) and AFBH treated (Group IV) showed significant increased levels (P<0.001). This was compared with Group I and Group II. These results are shown in the Table 5.3. After 8 weeks the changes in the lipid profile of experimental animals was observed. The levels of total cholesterol, triglycerides, LDL cholesterol was significantly increased (P<0.001) in Group II (HFD fed rats or obesity induced rats) and was compared with
Group I animals. This result is shown in the Table 5.3. Group III (EEBH treated rats), Group IV (AFBH treated rats) and Group V animals were found to

The changes in the lipid parameters like Total cholesterol, triglycerides, LDL, VLDL also significantly decreased, while comparing the group III with Group IV animals. When comparing Group III with Group IV animals, Group IV animals showed significant result than EEBH treated animals.

Table 5.3: Effect of AFBH and EEBH on Lipid profile, Atherogenic index in Serum of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>111 ± 3.60</td>
<td>218 ± 6.08 a*</td>
<td>170 ± 5.00 a<em>b</em></td>
<td>134 ± 3.51 a<em>b</em></td>
<td>150.33 ± 5.03 a<em>b</em></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>93.33 ± 2.08</td>
<td>187.66 ± 7.23 a*</td>
<td>149.33 ± 5.03 a<em>b</em></td>
<td>118.00 ± 2.00 a<em>b</em></td>
<td>135 ± 4.04 a<em>b</em></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>56.66 ± 1.15</td>
<td>30.00 ± 2.00 a*</td>
<td>36.00 ± 2.00 a<em>b</em></td>
<td>46.00 ± 1.00 a<em>b</em></td>
<td>41.00 ± 1.00 a<em>b</em></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>63.33 ± 1.52</td>
<td>150.46 ± 7.80 a*</td>
<td>104.13 ± 6.66 a<em>b</em></td>
<td>65.60 ± 2.94 aNSb*</td>
<td>82.26 ± 5.19 a<em>b</em></td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>18.60 ± 0.41</td>
<td>37.53 ± 1.44 a*</td>
<td>29.86 ± 1.00 a<em>b</em></td>
<td>23.6 ± 0.40 a<em>b</em></td>
<td>27.06 ± 0.61 a<em>b</em></td>
</tr>
<tr>
<td>Atherogenic index (A.I)</td>
<td>0.95 ± 0.02</td>
<td>6.28 ± 0.52 a*</td>
<td>3.73 ± 0.40 a<em>b</em></td>
<td>1.92 ± 0.11 a<em>b</em></td>
<td>2.66 ± 0.18 a<em>b</em></td>
</tr>
<tr>
<td>Coronary risk index (CRI)</td>
<td>1.95 ± 0.02</td>
<td>7.28 ± 0.52 a*</td>
<td>4.73 ± 0.40 a<em>b</em></td>
<td>2.92 ± 0.11 a<em>b</em></td>
<td>3.66 ± 0.18 a<em>b</em></td>
</tr>
<tr>
<td>HDL/LDL</td>
<td>0.93 ± 0.008</td>
<td>0.20 ± 0.023 a*</td>
<td>0.34 ± 0.041 a<em>b</em></td>
<td>0.70 ± 0.03 a<em>b</em></td>
<td>0.50 ± 0.04 a<em>b</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group

Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: *- P < 0.001; #- P < 0.01; @- P < 0.05,
NS-Non significant.
Comparison: a-as Compared with Group I; b-as Compared with Group II.
Retnasamy and adikay, 2014 studied the effect of *Hiptage madablota* Gaertn on high fat diet fed rats. These results correlate with our results. High fat diet showed significant increase in the level of Total cholesterol, triglycerides, LDL, VLDL and also decreased level of HDL.

Kameshwaran *et al.*, 2013 and Supriya et al., 2012 reported the same results. The level of triglycerides, total cholesterol, LDL and VLDL level increased and HDL level is decreased in high fat diet fed animals and extract treated showed significant reduction of these lipids and increase in the level of HDL.

Similar results have been reported by Norazmir and Ayub, 2010 in high fat diet fed rats and rats treated with pink guava puree extract.

**Coronary risk index and atherogenic index**

Coronary risk index and atherogenic index have been used as a marker for atherosclerosis. The CRI and AI are increased during coronary condition.

The atherogenic index and coronary risk index is shown in Table 5.3. There was significant increase (P<0.001) of atherogenic index and coronary risk index. The atherogenic index and coronary risk index was significantly decreased (P<0.001) in EEBH treated (Group III) and AFBH treated (Group IV) animals. This was compared with Group II and Group I animals. When comparing all the other groups AFBH treated animals showed best result in reducing the AI and CRI.

The elevated levels of both atherogenic index and coronary risk index in high fat diet induced obesity animals were changed by oral administration of hydro alcoholic leaf extracts of *Shorea robusta* (Supriya *et al.*, 2012).

The ethanolic extracts of roots of *Hiptage madablota* significantly improved the atherogenic index and coronary risk index in high fat diet fed animals as reported by Retnasamy and adikay, 2014.

**Free fatty acids and Phospholipids**

The elevation of serum free fatty acids and phospholipids are shown in of HFD fed rats. Table 5.4 shows the changes in free fatty acids and phospholipids in HFD fed rats. There was a significant increase (P<0.001) in serum of Group II rats caused due to hyperlipidemia, when compared to the corresponding control group (Group I).
Group III and Group IV animals showed significant decrease (P<0.001) in free fatty acids and phospholipids. This was compared with Group I and Group II animals. From the result Group IV animals showed significant reduction of free fatty acids and phospholipids.

Achuthan and Padikkala, 1997 stated that high cholesterol diet fed rats showed increased level of phospholipids. The high fat diet induced animals showed increased levels of phospholipids and when treated with *Alpina galangal* and *Kaempferria galangal* showed decreased level of phospholipids.

Hypercholesterolemia induced rats showed increased levels of Phospholipids and free fatty acids. High fat diet rats treated with abana extract showed decreased level of free fatty acids and phospholipids. This result was reported by Pragda et al., 2012.

High cholesterol fed diet showed increased level of free fatty acids. High fat diet fed animals showed increased level of free fatty acids and it was reported by Jegadeesh et al., 2014.

### Table 5.4: Shows the level of Free fatty acids and Phospholipids in serum of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids(mg/dl)</td>
<td>15.23 ± 1.11</td>
<td>29.48 ± 1.26a*</td>
<td>25.16 ± 1.69a<em>b</em></td>
<td>± 17.67 ± 1.29a<em>b</em></td>
<td>21.54 ± 1.29a<em>b</em></td>
</tr>
<tr>
<td>Phospholipids(mg/dl)</td>
<td>110.33 ± 8.59</td>
<td>250 ± 8.94a*</td>
<td>211.33 ± 11.67a<em>b</em></td>
<td>± 128.66 ± 7.22a<em>b</em></td>
<td>188.00 ± 5.58 a<em>b</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group

- **Group I** - Control animals, **Group II**- HFD fed animals
- **Group III** - Obesity induced animals treated with EEBH (300mg/kg body wt), **Group IV** - Obesity induced animals treated with AFBH (100mg/kg body wt), **Group V** - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: *- P < 0.001; # - P < 0.01.
Comparison: a-as Compared with Group I; b-as Compared with Group II.

Glucose, insulin level and HOMO IR
The consumption of HFD leads to obesity, fat accumulation in body and disturbance in glucose and lipid homeostasis. High fat diet lowers the fat uptake but also suppresses hepatic glucose production stimulated by insulin leading to insulin resistance as well as hyperglycaemia (Oakes et al., 1997).

The concentration of plasma insulin and glucose present in the HFD fed rats and is shown in Table 5.5. The levels of glucose, insulin and homo IR were significantly increased (P<0.001) in Group II animals when compared with Group I animals. The drug treated EEBH and AFBH was significantly less (P<0.001) when compared to Group II animals. AFBH treated groups showed better result in decreasing the insulin and glucose compared to the EEBH treated HFD fed animals.

The concentration of glucose, insulin and HOMO IR also decreased when treated with AFBH than EEBH.

The increased glucose level showed in the HFD fed rats are reported by Malik and Sharma 2011. They showed that high fat diet fed animals treated with zingiber officinale exert better activity in reducing glucose level.

The same results were observed with both AFBH and EEBH treated rats.

High fat diet fed rats showed rapid weight gain in rodents, mild hyperglycemia and hyper insulinenia to gather with reduced glucose disappearance rate (Srinivasan et al., 2004).

The increase in adipose tissue fat deposits, results in obesity and the ability of insulin to stimulate glucose transport and metabolism in adipocytes and skeleton muscle is impaired resulting in insulin resistance (Reaven, 1995). Impairment in insulin sensitivity led to dyslipidemia (Reaven, 2005).

Anyanwu, 2014 proved that the concentration of glucose and insulin is increased in high fat diet fed rats. In case of high fat diet groups treated with Sphenocentrum jollyanum pierre showed decreased level of glucose and insulin concentration.

Abo-Elmatty, 2011 determined the level of insulin in serum. Obesity induced rats showed increased level of insulin, glucose and HOMO IR and this was compared with topiramate treated obese rats. These rats showed decreased level of insulin, glucose and HOMO IR.
Table 5.5: Shows the level of Glucose and Insulin in Serum of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose(mg/dl)</td>
<td>83.33 ± 4.93</td>
<td>152.00 ± 6.08a*</td>
<td>105.33 ± 2.08a<em>b</em></td>
<td>99 ± 2.64a<em>b</em></td>
<td>91.00 ± 1.73a@b*</td>
</tr>
<tr>
<td>Insulin(µU/ml)</td>
<td>11.12 ± 0.29</td>
<td>19.10 ± 0.10a*</td>
<td>12.72 ± 0.10a<em>b</em></td>
<td>12.22 ± 0.15a<em>b</em></td>
<td>11.98 ± 0.12a<em>b</em></td>
</tr>
<tr>
<td>Homo IR</td>
<td>2.28 ± 0.13</td>
<td>7.16 ± 0.20a*</td>
<td>3.17 ± 0.08a<em>b</em></td>
<td>3.11 ± 0.12a<em>b</em></td>
<td>2.69 ± 0.03a<em>b</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group

- Control animals, Group II- HFD fed animals (Obesity induced animals),
- Obesity induced animals treated with EEBH (300mg/kg body wt),
- Obesity induced animals treated with AFBH (100mg/kg body wt),
- Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: *- P < 0.001; # - P < 0.01; @ - P < 0.05.
Comparison: a-as Compared with Group I; b-as Compared with Group II.

Total protein, albumin and Globulin level in serum of experimental animals

Table 5.6 reveals the changes of total protein and albumin levels in serum sample of control and experimental animals. Albumin and total proteins were significantly decreased in (P<0.001) in Group II obesity induced animals when compared to the total protein levels of Group I animals. Group III and Group IV animals showed significant increase in the level of total protein and albumin. This result is also compared to the normal control animals. AFBH treated animals showed significant result when compared with EEBH treated animals.

The globulin level was increased in Group II animals and Group III and Group IV animals showed that significant decreased level of globulin. These results are showed in Table 5.6.

The reports of Nuhu Alam, 2011 showed that levels of total protein and albumin in high fat diet fed rats and after treated with specific extracts like *pleurotus*
*citrinopileatus* showed significantly increased level of total protein and albumin and high fat diet fed rat showed significantly decreased level of total protein and albumin.

**Enzymatic markers in serum**

The animals fed with high cholesterol diet result in elevated serum or tissue cholesterol level. This causes metabolic disturbances; exogenous hypercholesterolemia causes the fat deposition in the liver and decreased hepatocyte production. This causes steatosis of liver due to the intracellular accumulation of lipids. High fat diet fed rats induces the production of free radicals. This in turns leads to the oxidative stress.

**Table 5.7** represents the activities of marker enzyme such as SGOT (AST), SGPT (ALT), ALP, LDH, CK and lipase in serum samples of control and experimental animals. The activities of SGOT, SGPT, ALP, CK, lipase and LDH showed significant increase (P<0.001) in Group II animals when compared to that of Group I animals. These marker activities are significantly decreased (P<0.001) in both EEBH and AFBH treated (Group III and IV) animals as compared to Group II animals. Liver enzyme marker level is also significantly decreased in Group IV animals. AFBH treated animals showed better result than EEBH treated animals.

Serum glutamate oxaloacetate transaminase (SGOT) also called as aspartate transaminase (AST) is a transaminase enzyme. This is clinically used as a marker for

**Table 5.6: Shows the level of Protein, Albumin and Globulin in Serum of experimental animals**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein(g/dl)</td>
<td>6.62 ± 0.01</td>
<td>5.99 ± 0.01 a*</td>
<td>6.32 ± 0.02a b*</td>
<td>6.53 ± 0.05a b*</td>
<td>6.40 ± 0.01a b*</td>
</tr>
<tr>
<td>Albumin(g/dl)</td>
<td>4.36 ± 0.01</td>
<td>3.63 ± 0.05 a*</td>
<td>3.98 ± 0.02a b*</td>
<td>4.26 ± 0.05a b*</td>
<td>4.15 ± 0.01a b*</td>
</tr>
<tr>
<td>Globulin(g/dl)</td>
<td>2.26 ± 0.02</td>
<td>2.36 ± 0.05 a@</td>
<td>2.44 ± 0.01a b NS</td>
<td>2.27 ± 0.02a NS b @</td>
<td>2.25 ± 0.05a NS b @</td>
</tr>
</tbody>
</table>
Each Value is expressed as mean ± SD for six animals in each group

- Control animals, Group II- HFD fed animals (Obesity induced animals),
- Obesity induced animals treated with EEBH (300mg/kg body wt),
- Obesity induced animals treated with AFBH (100mg/kg body wt),
- Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.

Table 5.7: Shows the activities of enzyme markers AST, ALT, ALP, LDH, CK and Lipase in Serum of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>120.33 ± 0.57</td>
<td>179.66 ± 9.07a*</td>
<td>114.66 ± 3.51a<em>NSb</em></td>
<td>115.66 ± 2.51a<em>NSb</em></td>
<td>128 ± 2.64a<em>NSb</em></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>55 ± 1.00</td>
<td>91.66 ± 2.08 a*</td>
<td>63.06 ± 1.40a<em>NSb</em></td>
<td>57.66 ± 0.5751a<em>NSb</em></td>
<td>69.66 ± 1.52a<em>NSb</em></td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>161.33 ± 1.52</td>
<td>251.66 ± 2.08a*</td>
<td>182.66 ± 2.51 a<em>NSb</em></td>
<td>173.66 ± 3.21 a<em>NSb</em></td>
<td>216.66 ± 3.05 a<em>NSb</em></td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>426.66 ± 16.64</td>
<td>673 ± 8.80a*</td>
<td>570 ± 19.49a<em>NSb</em></td>
<td>445 ± 11.83a<em>NSb</em></td>
<td>516 ± 6.83a<em>NSb</em></td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>411 ± 9.30</td>
<td>643.33 ± 13.66a*</td>
<td>569.66 ± 8.96 a<em>NSb</em></td>
<td>436.66 ± 10.32 a<em>NSb</em></td>
<td>528.33 ± 9.30 a<em>NSb</em></td>
</tr>
<tr>
<td>Lipase (IU/L)</td>
<td>649 ± 13.05</td>
<td>765.16 ± 13.51a*</td>
<td>716.83 ± 6.96 a<em>NSb</em></td>
<td>676.33 ± 4.45 a<em>NSb</em></td>
<td>688.33 ± 5.16 a<em>NSb</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group

- Control animals, Group II- HFD fed animals (Obesity induced animals),
- Obesity induced animals treated with EEBH (300mg/kg body wt),
- Obesity induced animals treated with AFBH (100mg/kg body wt),
- Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.
Comparison: a-as Compared with Group I; b-as Compared with Group II.

Liver. These enzymes are involved in the transfer of amino group from alanine to α-ketoglutarate. Alkaline phosphatase is the enzyme involved in the removal of phosphate groups from the molecules. An elevated level of aspartate transaminase level is not specific for liver marker but also used as cardiac marker (Preussnner Harold, 1998). Lactate dehydrogenase (LDH) is the enzyme involved in the conversion of pyruvate to lactate under anaerobic conditions. Elevated level of LDH indicate liver damage. LDH also act as marker for myocardial infarction (Shinde and Goyal, 2003).

The reports of Norazmir and Ayub, 2010 stated that elevated level of ALP, ALT, AST, CK and LDH in serum of high fat diet fed animals and were brought to near normal when treated with pink guava puree.

Mohan and Gayathri 2013 have suggested that the level of ALT and AST is increased in high fat diet fed rats. HFD fed rats when treated with Hibiscus cannabinus revealed that these enzyme activities are near normal.

Athus et al., 2014 also reported that the activity of ALP, ALT and AST increased in high fat diet fed rat and HFD treated with Cyperus rotundus tuber extract exerts lesser activity of these enzymes.

Malik and Sharmaa 2011 showed that administration of Zingiber officinale decreased the activity of AST and ALT and this was compared with high fat diet fed rats. High fat diet fed rats exerts an increased level of AST and ALT.

Prabha et al., 2013 also showed the effect of methanolic extract of Gardenia gymnifera linn. on high fat diet fed rats. High fat diet fed rats showed increased level of CK and LDH but Gardenia gymnifera lin. treated rats showed significantly decreased activity of CK and LDH.

Lipase

Dietary lipids cannot be absorbed from the intestinal linings without undergoing hydrolysis by lipase. Inhibition of lipase activity leads to decrease in intestinal lipid digestion and absorption (Hamden et al., 2010).

The increased level of lipase in high fat diet fed rats was inferred by Kumar et al., 2014. lipase enzyme is involved in the degradation of triglycerides into free fatty acid and glycerol. Fenugreek treated rats showed decreased activity of lipase activity.
**Homocysteine**

Homocysteine is non-essential amino acid. Homocysteine is formed from demethylation of methionine. Homocysteine can be remethylated into methionine by vitamin B12 dependent methionine synthase and methyl tetra hydrofolate. Homocysteine and hypercholesterolemia is associated with risk factor of cardio vascular disease ([Herrmann et al., 2004](#)). The level of homocysteine increases in the blood and causes cholesterol to oxidized low density lipoprotein, which can damage the arteries by causing plaque inside artery wall ([McCully, 1996](#)).

Homocysteine level is significantly increased (P<0.001) in Group II animals. This result was compared with Group I animals. These results are shown in the Figure 5.7. Homocysteine level of high fat diet fed rats treated with EEBH and AFBH showed significant decrease (P<0.001). Orlistat treated animals showed significant decrease (P<0.001). These results were compared with group I and group II animals. Comparing all the groups AFBH showed better result in reducing the homocysteine level, which was near normal when compared to control animals.

Kapoor et al., 2008 explained about the level homocysteine in curcumin treated, methionine induced hyperlipidemia and hyperhomocysteinemia. High fat diet fed rats showed increased level of homocysteine when compared with normal control. High fat diet fed rat treated with curcumin showed decreased level of homocysteine.

The present study reveals that high fat diet treated with AFBH extract showed significant result than EEBH treated animals.

**Leptin and Adiponectin**

Adipocytes secrete various adipocytokines such as tumor necrosis factor-α, leptin, adiponectin, and resistin. Adiponectin plays an important role in insulin sensitivity and fatty acid oxidation, and adiponectin levels are negatively correlated with body fat mass, and serum glucose, insulin, and triglyceride levels ([Arita et al., 1999](#)).

Figure 5.8 showed that changes in the leptin and adiponectin level in serum and control experimental animals. The levels of leptin was significantly increase (P<0.001) in Group II. Group III and Group IV animals showed significant decrease (P<0.001) level of leptin. This results were compared to the Group II and Group I animals. AFBH treated animals showed best result in reducing leptin than EEBH treated animals.
Each Value is expressed as mean± SD for six animals in each group
Group I - Control animals,
Group II - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
*-P < 0.001; #- P < 0.01; @- P < 0.05, NS-Non significant.
Comparison:
a-as Compared with Group I; b-as Compared with Group II.

Adiponectin levels of experimental rats are shown in Figure 5.8. The level of adiponectin level was significantly decreased (P<0.001) in Group II animals. The results were compared with Group I (normal) control animals. The adiponectin level was significantly increased in Group III (EEBH) treated and Group IV (AFBH) treated. These results were compared with Group I and Group II animals.

The current study reported that AFBH treated animals showed significantly increased level of adiponectin but EEBH treated animals showed slight elevation of adiponectin level. AFBH treated animals showed good result.
Adiponectin plays an important role in the regulation of lipid metabolism and insulin sensitivity. Adiponectin also has an anti-inflammatory and antiatherogenic property. Adiponectin exhibits insulin sensitizing effect via AMPK activation in peripheral tissues that led to the stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and suppression of glucose production in liver. Circulating adiponectin levels are negatively correlated with obesity, particularly visceral obesity and insulin resistance (Blaslov et al., 2013).

Leptin is a hormone secreted from adipose tissue and regulates appetite, adiposity and increases the adipose tissue weight, serum leptin levels also (Aizawa-Abe et al., 2000). Hyperlipidemic condition tends to increase the leptin resistance and increases the oxidative stress caused due to reactive oxygen species in accumulated fat. This leads to the elevated adipose nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that leads to uncontrolled production of leptin (Furukawa et al., 2004).

Kumar et al., 2014 reported that the levels of leptin increased in HFD fed rats. Fenugreek treated HFD rats ameliorates leptin level. Adiponectin level in the high fat diet fed rats showed decreased levels. This was compared with control rats. The fenugreek treated rats tend to increase the adiponectin level.

Mahmoud Hassan El-Bidawy et al., 2013 showed that increased level of adiponectin in HFD treated with cinnamon. This was compared with control and High fat diet rats. High fat diet fed group rats showed significantly decreased level of adiponectin.
Each Value is expressed as mean ± SD for six animals in each group
Group I - Control animals,
Group II - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
* - P < 0.001; # - P < 0.01; @ - P < 0.05.
Comparison:
 a-as Compared with Group I; b-as Compared with Group II.

Dohaekseunggi tang extract showed increased levels of adiponectin in high fat diet fed rats. Adiponectin level is diminished in high fat diet induced rats and were proved by Sung et al., 2014. Leptin level is increased in HFD fed rats and HFD treated Dohaekseunggi tang extract decreases the leptin level.

High fat diet fed rats exerts increased levels of leptin. When compared with normal and HFD fed rats treated with Sphenocentrum jollyanum pierre showed lower level of leptin as reported by Anyanwu in 2014.
Hence these results support the results obtained with EEBH and AFBH treated HFD fed rats.

**Apolipoprotein B**

Apo-B is synthesized in liver and indicates the amount of atherogenic lipoproteins in plasma or hepatic tissue, and it is considered as a better predictor of coronary heart disease (*Walldius et al., 2001*). Obesity is an independent risk factor for cardiovascular disease (CVD), via its influence on other known risk factors such as dyslipidemia and hypertension.

Apo B-100 is the major protein component of very low, intermediate and low density lipoproteins (VLDL, IDL and LDL). These particles are involved in cascade of triglyceride rich VLDL and released from the liver and converted into cholesterol rich LDL. Abnormal Apolipoprotein B metabolism leads to hyperlipidemia condition, which in-turn is involved in the development of coronary heart disease (*Packard et al., 2000*).

Apo-B is the lipoprotein that is responsible for transport of lipids. Apolipoprotein B level was significantly decreased (P<0.001) in EEBH and AFBH treated HFD fed rats. High fat diet fed rats showed increased apolipoprotein B level (P<0.001). This result is shown in Figure 5.9. Group III and Group IV animals showed decreased level of apolipoprotein B and this result was compared with Group I and Group II. AFBH treated animals showed best result in reducing the Apo B than EEBH treated animals. Apo-B levels in Group IV animals are similar to that of Group I animals. So AFBH showed better result and is found to reduce Apo-B.

*Asgary et al., 2013* reported that sesamum indicum treated high cholesterolemic diet fed animals showed decreased Apolipoprotein B. This was compared with high cholesterol diet fed rabbit which showed increased level of Apolipoprotein B.
Figure 5.9: Shows the level of Apolipoproteins

Each Value is expressed as mean ± SD for six animals in each group

Group I  - Control animals,
Group II  - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V  - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
* - P < 0.001.

Comparison:
a-as Compared with Group I; b-as Compared with Group II.

Kumar et al., 2014 reported that the level of Apolipoprotein is increased in HFD fed rats and fenugreek seed treated rats showed decreased level of apolipoprotein B.

Similar result was explained by Ramyasai et al., 2013. High fat diet fed rats treated with methanolic extract of Couroupita guianensis aubl flower showed significant reduction of Apo-B as compared with normal and high fat diet fed animals.

Assay of Pancreatic lipase activity

Pancreatic lipase or triacylglycerol acyl hydrolase is the lipolytic enzyme synthesized and secreted by pancreas. Lipase plays a key role in digestion of
triglycerides, phospholipids and is responsible for the hydrolysis of dietary fat. Pancreatic lipase inhibition is one of the widely studied mechanisms used to determine the potential efficacy of natural products as antiobesity agents (Thomson et al., 1997).

Figure 5.10 shows that the \textit{in vivo} pancreatic lipase activity in experimental animals. The pancreatic lipase activity is increased (P<0.001) in the Group II animals when compared with the Group I animals. The pancreatic lipase activity in Group III and Group IV animals showed significantly decreased value (P<0.001). This result was compared with both Group I and Group II experimental animals. AFBH treated high fat diet fed animals showed better result than EEBH treated animals.

This result proved that AFBH showed better pancreatic lipase inhibition activity than EEBH treated HFD fed rats. So AFBH act as potent inhibitor of pancreatic lipase and hence it could act as a potent antiobesity agent.

Deshpande et al., 2013 reported the pancreatic lipase activities. The pancreatic lipase activities in obese rats treated with \textit{Ziziphus maruritiana} showed decreased activity of pancreatic lipase. This was compared with obese rat and normal animals.

**Lipid peroxidation in the Serum**

The intra cellular antioxidants are involved in scavenging of the reactive oxygen species. The generation of large amounts of reactive oxygen species can be reduced by the intracellular antioxidant defences which may cause protein modification and DNA breaks (Hiroi et al., 1999).

Lipid Peroxidation in biological membranes can cause an impairment of membrane function and decrease the fluidity and has been associated with a number of disease states from atherosclerosis and Ischaemia- reperfusion injury to hyperoxic lung
Each Value is expressed as mean ± SD for six animals in each group

Group I - Control animals,
Group II - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
*P < 0.001.

Comparison:
a-as Compared with Group I; b-as Compared with Group II.

injury. Lipid peroxidation is initiated by the attack of an acyl side chain on a fatty acid by any chemical species. Increased levels of peroxides are observed in the plasma due to the consequence of the increased free radical production and the lipid peroxides are liberated into circulation due to pathological changes. Lipid peroxidation acts as a secondary mediator of oxidative stress both extracellularly and intracellularly, specifically to DNA. Lipid peroxidation products such as thiobarbituric acid reactive substances, hydroperoxides levels as well as protein markers of oxidative damage of reactive oxygen species are observed (Olusi, 2002).
Figure 5.11 shows LPO level in the serum of experimental animals. LPO level in serum is significantly increased (P<0.001) in Group II animals when compared with Group I (normal) control. Group III and Group IV animals showed significantly decreased level of LPO (P<0.001). This result was compared with Group I and Group II animals. While comparing Group III and Group IV animals, Group IV animals showed good result in lowering lipid peroxidation than Group III animals.

**Lipid peroxidation (LPO) in Liver tissue**

Figure 5.12 shows the LPO level in the liver of experimental animals. LPO level in liver is significantly increased (P<0.001) in Group II animals when compared with Group I animals. Group III and Group IV animals showed significant decrease (p<0.001) in LPO in liver. This was compared with Group II and Group I animals. Comparing all the other groups Group IV showed promising result in lowering lipid peroxidation.

Sikder et al., 2014 explained about oxidative stress due to high fat diet. Lipid accumulation causes increased production of LPO. High fat diet fed rats treated with quercetin and β Sitosterol showed decreased level of LPO production.

Athesh et al., 2014 reported the increased level of LPO in liver of high fat diet fed rats and HFD fed rats treated with Cyperus rotundus showed decreased level of LPO in liver.

The same results have been reported by Lee et al., 2011; Muthu et al., 2010 and showed that high fat diet fed animals treated with Portulaca oleracea powder and Borreria hispida showed significantly reduced the levels of LPO in liver of experimental animals.
Figure 5.11: Shows the levels of Lipid peroxides in serum of experimental animals

Each Value is expressed as mean± SD for six animals in each group

Group I - Control animals,
Group II - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
*-P < 0.001; #- P < 0.01; @- P < 0.05, NS-Non significant.

Comparison:
a-as Compared with Group I; b-as Compared with Group II.
Figure 5.12: Shows the levels of Lipid peroxides in Liver of experimental animals

Each Value is expressed as mean± SD for six animals in each group

Group I - Control animals,
Group II - HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance:
* - P < 0.001; # - P < 0.01; @ - P < 0.05.

Comparison:

a-as Compared with Group I; b-as Compared with Group II.

Antioxidant parameters in Serum and Liver

Oxidative stress was found to be associated with obesity. There is a growing awareness that obesity is a prime risk factor for the development of dyslipidemia profile and that oxidative stress may play a role in various adverse effects of obesity (Diniz et al., 2005). Oxidative stress is an imbalance between tissue free radicals, and
antioxidants and may be key this mechanism underlying obesity related co-morbidities. This can be maintained by the level of antioxidant supplement.

Table 5.8 represented the enzymatic antioxidant level of serum in experimental animals. The activity of SOD, catalase and GPX are significantly decreased (P<0.001) in Group II when compared with Group I animals. Group III and Group IV animals showed a significant increase of SOD, catalase and glutathione peroxidase activities when compared with Group II animals. Group IV (AFBH) showed better result than EEBH treated animals.

Enzymatic antioxidants in liver of experimental animals is also shown in the Table 5.8. The SOD, catalase and GPX are significantly decreased (P<0.001) in Group II when compared with Group I normal animals. SOD, catalase (CAT), glutathione peroxidase (GPX) levels were significantly increased in Group III, IV and Group V animals. This result was compared with normal (control) group and HFD fed rats group. From this result it can be showed that AFBH treated animals showed better result is increasing the activities of enzymatic antioxidant than EEBH treated animals.

The pink guava puree restored the levels of SOD and GPx levels in serum of high fat diet induced obesity animals (Norazmir et al., 2010). Aqueous tuber extract of Cyperus rotundus L supplementation restored the hepatic SOD, CAT and GPx activities in high fat diet induced group of animals (Athesh et al., 2014).

Non enzymatic antioxidants in Serum and Liver

Table 5.9 showed the non-enzymatic antioxidants such as Glutathione, Vitamin C and Vitamin E level in liver and serum of experimental animals. Group II animals exhibited decreased level of Glutathione (GSH), Vitamin C and Vitamin E (P<0.001). The results were compared with Group I animals. The Group III and Group IV animals showed significantly increased level of this non-enzymatic antioxidants in serum and
### Table 5.8: Shows the enzymatic antioxidants of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymatic antioxidants in Serum of experimental animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD(U/mg of protein)</td>
<td>4.75 ± 0.02</td>
<td>3.04 ± 0.03 a*</td>
<td>3.53 ± 0.03 a<em>b</em></td>
<td>3.73 ± 0.14 a<em>b</em></td>
<td>3.02 ± 0.14 a*b NS</td>
</tr>
<tr>
<td>Catalase (μmoles of H2O2 utilized/min/mg of protein)</td>
<td>34.66 ±1.52 a*</td>
<td>23.88 ± 1.83 a*</td>
<td>28.63 ± 0.28 a<em>b</em></td>
<td>30.50 ± 0.50 a<em>b</em></td>
<td>29.42 ± 0.43 a<em>b</em></td>
</tr>
<tr>
<td>GPx (μmoles of glutathione utilized/min/mg of protein)</td>
<td>3.76 ± 0.05</td>
<td>1.67 ± 0.16 a*</td>
<td>3.04 ± 0.12 a<em>b</em></td>
<td>3.68 ± 0.18 a<em>b</em></td>
<td>3.54 ± 0.02 a<em>b</em></td>
</tr>
<tr>
<td><strong>Enzymatic antioxidants in Liver of experimental animals</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SOD(U/g of tissue)</td>
<td>6.30 ± 0.05</td>
<td>2.33 ± 0.21 a*</td>
<td>3.16 ± 0.12 a<em>b</em></td>
<td>4.08 ± 0.02 a<em>b</em></td>
<td>3.68 ± 0.80 a<em>b</em></td>
</tr>
<tr>
<td>Catalase (μmoles of H2O2 utilized/min/mg of protein)</td>
<td>57.51 ± 2.18 a*</td>
<td>27.12 ± 1.18 a*</td>
<td>36.81 ± 1.73 a<em>b</em></td>
<td>45.27 ± 1.43 a<em>b</em></td>
<td>38.01 ± 1.01 a<em>b</em></td>
</tr>
<tr>
<td>GPx (μmoles of glutathione utilized/min/mg of protein)</td>
<td>4.46 ± 0.15</td>
<td>1.17 ± 0.06 a*</td>
<td>3.53 ± 0.25 a<em>b</em></td>
<td>4.07 ± 0.05 a<em>b</em></td>
<td>3.17 ± 0.06 a<em>b</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group.

- **Group I**: Control animals, **Group II**: HFD fed animals (Obesity induced animals),
- **Group III**: Obesity induced animals treated with EEBH (300mg/kg body wt),
- **Group IV**: Obesity induced animals treated with AFBH (100mg/kg body wt),
- **Group V**: Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: * - P < 0.001; # - P < 0.01; @ - P < 0.05, NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.
Table 5.9: Shows the Non enzymatic antioxidants of experimental animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non enzymatic antioxidants in Serum of experimental animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH (µg/mg of protein)</td>
<td>5.60 ± 0.10</td>
<td>3.66 ± 0.15 a*</td>
<td>4.13 ± 0.05 a<em>b</em></td>
<td>4.70 ± 0.10 a<em>b</em></td>
<td>4.85 ± 0.06 a<em>b</em></td>
</tr>
<tr>
<td>Vitamin-C (mg/dl)</td>
<td>2.67± 0.02</td>
<td>2.05 ± 0.04 a*</td>
<td>2.21 ± 0.01 a<em>b</em></td>
<td>2.51 ± 0.01 a<em>b</em></td>
<td>2.35 ± 0.01 a<em>b</em></td>
</tr>
<tr>
<td>Vitamin-E (mg/dl)</td>
<td>1.96 ± 0.15</td>
<td>0.88 ± 0.01 a*</td>
<td>1.34 ± 0.07 a<em>b</em></td>
<td>1.90 ± 0.01 a<em>b</em></td>
<td>1.70 ± 0.01 a<em>b</em></td>
</tr>
<tr>
<td><strong>Non enzymatic antioxidants in Liver of experimental animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH(µg/g of tissue)</td>
<td>43.50 ± 1.32</td>
<td>29.00 ± 1.00 a*</td>
<td>35.16 ± 1.04 a<em>b</em></td>
<td>41.06 ± 1.00 a<em>b</em></td>
<td>37.20 ± 1.05 a<em>b</em></td>
</tr>
<tr>
<td>Vitamin-C (mg/g of tissue)</td>
<td>1.40 ± 0.10</td>
<td>0.81 ± 0.04 a*</td>
<td>1.16 ± 0.05 a<em>b</em></td>
<td>1.38 ± 0.07 a<em>b</em></td>
<td>1.15 ± 0.20 a<em>b</em></td>
</tr>
<tr>
<td>Vitamin-E (mg/g of tissue)</td>
<td>5.60 ± 0.10</td>
<td>3.33 ± 0.15 a*</td>
<td>4.20 ± 0.11 a<em>b</em></td>
<td>4.82 ± 0.14 a<em>b</em></td>
<td>4.24 ± 0.16 a<em>b</em></td>
</tr>
</tbody>
</table>

Each Value is expressed as mean ± SD for six animals in each group

Group I - Control animals, Group II- HFD fed animals (Obesity induced animals),
Group III - Obesity induced animals treated with EEBH (300mg/kg body wt),
Group IV - Obesity induced animals treated with AFBH (100mg/kg body wt),
Group V - Obesity induced animals treated with orlistat (25mg/kg body wt).

Statistical significance: *- P < 0.001; # - P < 0.01; @ - P < 0.05,
NS - Non significant.

Comparison: a-as Compared with Group I; b-as Compared with Group II.

liver. This result was compared with Group I and Group II animals. Group IV (AFBH) showed better result than EEBH treated animals.
The GSH, SOD and Vitamin C are the main free radical scavenging antioxidants which play a key role in repairing the biological damages by free radicals (Cemek et al., 2008).

The pink guava puree restored the levels of SOD and GPx levels in serum of high fat diet induced obesity animals (Norazmir et al., 2010).

Sikder et al., 2014 observed that high fat diet fed animals treated with Quercetin and β-Sitosterol showed better result to increase the antioxidant level than high fat diet fed rats.

Muthu et al., 2010 explained about changes in the level of GSH, SOD, GPX in liver of high fat diet fed animals treated with methanolic extract of Borreria hispida. High fat diet fed animals showed reduced level of these enzymes but methanolic extract of Borreria hispida treated animals showed increased levels of antioxidants.

Similarly, decrease in liver SOD and CAT activities are caused due to obese mice fed with high fat diet and was increased by the administration of ethanolic root extract of Adenophora triphylla (Seong Eun lee et al., 2013).

**Histopathological analysis**

**Histopathology of Adipose tissue**

Plate A1-A5 depicts the histopathological changes in adipose tissue of control and experimental animals. The adipose tissue of control animals reveals normal histology (Group I). In Group II (HFD fed animals) showed enlarged adipocyte. Group III and Group IV animals showed reduced size of adipocyte size. Group V orlistat treated animals showed moderated level of adipocyte. The size of adipocyte is compared with normal and high fat diet fed rat.

Sung, 2014 stated that histology of liver by haetoxylin eosin staining. High fat diet fed rats showed significant increased adipocyte size due to the fat accumulation in adipocyte. Dohaekseunggi tang extract treated rats showed significant reduction of adipocyte size due to decreased level of lipid accumulation.

Malik and Sharma et al., 2011 reported that ethanolic root extracts of licorice has suppressed adipocyte size when compared to high fat diet fed animals. High fat diet
Plate A1-A5 shows the histopathology of adipose tissue of experimental animals

Plate A1: GROUP I

Plate A2: GROUP II

Plate A3: GROUP III

Plate A4: GROUP IV

Plate A5: GROUP V

The images are shown at 40X magnification.

- Plate A1 shows normal morphology of adipose tissue.
- Plate A2 shows enlargement of adipocyte. (HFD fed rats)
- Plate A3 shows slightly enlarged adipocyte. (HFD treated with EEBH (300mg/kg body wt))
- Plate A4 shows very slightly enlarged adipocyte. (HFD treated with AFBH (100mg/kg body wt))
- Plate A5 shows moderately enlarged adipocyte. (HFD treated with Orlistat (25mg/kg body wt))
fed animals showed increases in the clusters of nucleated cells in the white adipose tissue when compared with the control animals.

*Boesenbergia pandurata* administrated animals showed decreased clusters of small nucleated cells in the white adipose tissue as demonstrated by *Kim et al., 2012*.

*Ho et al., 2012* explained about the histological examination of adipose tissue in high fat diet fed group and reported that more number of adipocytes was found when compared with administration of germinated brown rice extract treated animals.

**Histopathology of Liver**

*Plate B1-B5* showed the histological analysis of liver of control and experimental groups. The control group showed the normal morphology of liver. Hepatocytes are clear broad with central vein at portal layer. Group II animals showed the steatosis of liver and also lipid accumulation was assessed by oil red O staining. This result was shown in *Plate C1-C5*. Group III animals showed reduced level of lipid accumulation, Group IV animals also showed reduced lipid content. Group II animals showed steatosis of liver when compared with Group III and IV. Group V showed slight steatosis of liver.

*Pragda et al., 2012* explained about histology of liver in hyperlipidemia rats. Group II rats showed centrilobular fatty degeneration, cloudy swelling and necrosis of hepatic cells.

Histology of liver changes may be due to lipid accumulation and fatty liver. The level of lipid accumulation in liver is reduced by poly herbal treatment and high fat diet fed rat showed steatosis of liver due to lipid homeostasis as reported by *Raj et al., 2012*.

*Khalid and Siddiqui, 2012* reported the effect of *Punarnava* root extract against high fat diet fed rats. High fat diet fed rats showed that there has been deposition of lipid in adipocytes. The Punarnava root extract showed decreased level of lipid accumulation in adipocytes.

Fatty changes were noted in the liver of high fat diet fed rats. Fat accumulation in the liver was reduced when treated with *Melothria maderaspatana* extract as reported by *Pandey et al., 2010*.
Plate B1-B5 shows the histopathology of Liver of experimental animals

Plate B1-GROUP I shows normal morphology of liver.
Plate B2-GROUP II shows steatosis of liver (HFD fed rats).
Plate B3-GROUP III shows less degree of steatosis of liver. (HFD treated with EEBH (300mg/kg body wt))
Plate B4-GROUP IV shows less degree of steatosis of liver. (HFD treated with AFBH (100mg/kg body wt))
Plate B5-GROUP V shows moderate steatosis of liver. (HFD treated with Orlistat (25mg/kg body wt))

The images are shown at 40X magnification.
Plate C1-C5 shows the histopathology of liver using oil red O staining of experimental animals

Plate C1-GROUP I
Plate C2-GROUP II
Plate C3-GROUP III
Plate C4-GROUP IV
Plate C5-GROUP V

The images are shown at 40X magnification.

- Plate C1 shows normal lipid accumulation of liver.
- Plate C2 shows more lipid accumulation of liver. (HFD fed rats)
- Plate C3 shows slight increase in lipid accumulation of liver. (HFD treated with EEBH (300mg/kg body wt))
- Plate C4 shows normal lipid accumulation of liver. (HFD treated with AFBH (100mg/kg body wt))
- Plate C5 shows slight lipid accumulation of liver. (HFD treated with Orlistat (25mg/kg body wt))
Kumar et al., 2014 suggested the same result when observed with high fat diet and reported that it increases the fat accumulation in liver. Fenugreek seed extract treated with high fat diet reduces the fat accumulation in hepatocytes.

Bhathena et al., 2011; Handayani et al., 2014 showed that diet with high fat content causes fat accumulation in hepatocytes and it was studied by using the haematological method of Oil red O staining.

**Histopathology of Heart**

Plate D1-D5 showed the histological analysis of heart of control and experimental groups of animals. Histological analysis showed no changes.

**Histopathology of Pancreas and Spleen:**

Histology of pancreas is shown in Plate E1-E5 and spleen is shown in Plate F1-F5. Both spleen and pancreas revealed the normal appearance with no significant changes in experimental groups.

Thus it may be inferred that AFBH and EEBH act as a potent antioxidant and antihyperlipidemic agent. Comparing EEBH with AFBH, AFBH showed better results in reducing the lipids than EEBH and also act as a potent anti-obesity agent. AFBH can ameliorate obesity and improve the glucose, lipid abnormalities and also hepatosteatosis.
Plate D1-D5 shows the histopathology of Heart of experimental animals

Plate D1: GROUP I  Plate D2: GROUP II  Plate D3: GROUP III
Plate D4: GROUP IV  Plate D5: GROUP V

The images are shown at 40X magnification.

Plate D1, D2, D3, D4 and D5 shows normal histology of heart
Plate E1-E5 shows the histopathology of Pancreas of experimental animals

The images are shown at 40X magnification.

- Plate E1, E2, E3, E4 and E5 shows normal histology of pancreas.
Plate F1-F5 shows the histopathology of Spleen of experimental animals

The images are shown at 40X magnification.

- Plate F1, F2, F3, F4 and F5 shows normal histology of spleen.