"ANTI-HYPERLIPIDEMIC ACTIVITY OF AERIAL PARTS OF *DELONIX ELATA* ON HIGH CHOLESTEROL DIET INDUCED HYPERLIPIDEMIA IN RATS"

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements for the Award of Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

BY

Ms. R. Sivagamasundari (Reg.No: 261526003)

Under the guidance of

Dr. C. Ronald Darwin., M.Pharm. Ph.D

Professor, Department of Pharmacology

DEPARMENT OF PHARMACOLOGY

MOHAMED SATHAK A.J. COLLEGE OF PHARMACY SHOLINGANALLUR,

CHENNAI – 600 119.

APRIL 2017

Ms. R. Sivagamasundari (Reg.no: 261526003)

II year-M.Pharm, Pharmacology

Department of pharmacology

Mohamed Sathak A.J. College of Pharmacy

DECLARATION OF THE CANDIDATE

I hereby declare that the thesis titled **"Anti-hyperlipidemic activity of aerial parts of Delonix elata on high cholesterol diet induced hyperlipidemia in rats"** submitted in partial fulfillment for the award of degree of **Master of Pharmacy** of the Tamil Nadu Dr. M.G.R. Medical university and carried out at Mohamed Sathak A.J. College of Pharmacy, Chennai, is my original and independent work done under the direct supervision and guidance of the in Pharmacology is a bonafide individual research work done by **Ms. R. Sivagamasundari (Reg.No: 261526003),** Chennai, under the guidance of **Dr. C. Ronald Darwin, M.pharm.,Ph.D, Department of Pharmacology** during the academic year 2016 – 2017. This thesis contains no material which has been accepted for the award of any degree or diploma of other universities.

Place : Chennai   **(Ms. R. Sivagamasundari)**

Date :

APPENDIX LIST OF FIGURES

ABBREVIATIONS

(Ob/Ob)	-	Obese
T2DM	-	Type 2 Diabetes Mellitus
ACTH	-	Adreno Corticotropic Hormone
ATP	-	Adenosine Tri Phosphate
AUC	-	Area Under The Curve
BM I	-	Body Mass Index
BP	-	Blood Pressure
BW	-	Body Weight
CAT	-	Catalase Activity
CHOD	-	POD –Cholesterol Oxidese /Peroxidese
CMC	-	Carboxy Methyl Cellulose
CNS	-	Catecholaminergic or Serotonergic
CT	-	Computed Tomography
CVD	-	Cardiovascular Disease
EGCG	-	Epigallocatechin Gallate
GH	-	Growth Hormone
GPO	-	Glycerophoshate Oxidase
GSH	-	Growth Stimulating Hormone
HDL	-	High Density Lipoprotein
IGF	-	Insulin -Like Growth Factor
LDL	-	Low Density Lipoprotein

MC4R	-	Type 4-Melanocortine Receptor
NALD	-	Nonalcoholic Liver Disease
NASH	-	Nonalcoholic Steatohepatitis
NHANES	-	National Health And Nutrition Examination Surveys
OECD	-	Organization for Economic Co-operation and Development
PC	-	Proenzyme Convertase
POMC	-	Pro OpiMelanocortin
PPAR	-	Peroxisome proliferatoractivated receptor
S1p and S2P	-	Site-1 AND 2 Protease
SCAP	-	SREBP Cleavage Activating Protein
SOD	-	Superoxide Dismutase
SREBP	-	Sterol Regulatory Element Binding Protein 1 and 2
TC	-	Total Cholesterol
TG	-	Triglycerides
TNF	-	Tumor Necrosis Factor
IL	-	Inter Leukin
TSH	-	Thyroid Stimulating Hormone
VFO	-	Visceral Fact Obesity
VLDL	-	Very Low Density Lipoprotein
WHO	-	World Health Organization
NIH	-	National Institute of Health

I DEDICATED THIS PROJECT TO MY HUSBAND AND MY SON

1. INTRODUCTION

1.1 Obesity

Obesity is a term applied to excess body weight with an abnormally high proportion of body fat. Thermodynamically speaking, imbalance between energy intake (feeding) and energy expenditure (physical activity) leads to obesity (Pilch & Bergenhem, 2006). Obesity has emerged as one of the major health concerns in the 21st century. The morbidity and mortality associated with obesity continue to increase and is one of the leading causes of preventable death (Barness, Opitz & Gilbert- Barness, 2007). Development of obesity is, however, more complicated than that; sedentary life style, genetic factors, medical illness, microbiological aspects, social factors and neurobiological mechanisms are also involved (Sugerman, 2005; Bleich & Cutler, 2008).

Obesity has reached epidemic proportions globally, with more than 1 billion adults overweight - at least 300 million of them clinically obese - and is a major contributor to the global burden of chronic disease and disability. Often coexisting in developing countries with under-nutrition, obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups (Global Strategy on Diet, Physical Activity And Health, WHO 2003). Increased consumption of more energy-dense, nutrient-poor foods with high levels of sugar and saturated fats, combined with reduced physical activity, have led to obesity rates that have risen three-fold or more since 1980 in some areas of North America, the United Kingdom, Eastern Europe, the Middle East, the Pacific Islands, Australia and China. The obesity epidemic is not restricted to industrialized societies; this increase is often faster in developing countries than in the developed world (Global Strategy on Diet, Physical Activity And Health, WHO 2003). A growing public health concern is that the prevalence of obesity among children aged 6–19 is up to 16.5% in the USA (Hedley *et. al,* 2004) and has also increased in Europe, Asia, Africa and South American countries (Antipatis & Gill, 2001). Despite increased attention given to overweight and obesity by every major body concerned with public health, including the National Institutes of Health (NIH) (National Task Force, 1994) the Centers for Disease Control (Mokdad *et. al.,* 2001),

the United States Department of Agriculture (Freedman King & Kennedy, 2001) and the World Health Organization (WHO, 2000) primary and secondary prevention efforts have generally been disappointing.

Obesity impacts many facets of society. For example, it is economically costly to society (World Health Organization, 1998) increases mortality rate (Allison, Fontaine, Manson, Stevens & VanItallie, 1999) reduces quality of life (Fontaine, Bartlett & Barofsky, 2000) and increases the risk of various morbidities (Billington, 2000). Extreme obesity has been estimated to truncate the lifespan of young adults by 5–20 years (Fontaine, Bartlett & Barofsky, 2000). The medical problems caused by obesity begin at the head and end at the toes and involve almost every organ in between. Several of these problems contribute to the earlier mortality associated with obesity and include coronary artery disease, severe hypertension that may be refractory to medical management, impaired cardiac function, adult-onset (type 2) diabetes mellitus, obesity hypoventilation and sleep apnea syndromes, cirrhosis, venous stasis and hypercoagulability with an increased risk of pulmonary embolism, and necrotizing panniculitis (Sugerman, 2005).

The association of obesity with T2DM (Type 2 DM) has been observed in comparisons of different populations and within populations (West & Kalbfleisch, 1971; Joslin, 1921). Approximately 10% of the obese population develop T2DM (Harris, Flegal & Cowie, 1998). This may be because of "glucotoxicity" (expression of glucose allostasis and increase in „allostatic load), "lipotoxicity" fatty acid supply to peripheral tissues impairs glucose uptake and storage in the muscle (Boden, 1997; Balasse & Neef, 1973; Gomez, Jequier, Chabot, Buber, & Felber, 1972; Kumar, Boulton, Beck-Nielsen, 1996) and adipokines (adipose tissue) hormones that are secreted by the adipose tissue (TNF-a, IL-6, complement C3, MIF, adiponectin) are associated with insulin resistance, often independently of the degree of adiposity (Straczkowski, *et. al.*, 2002; Weyer, 2000; Weyer, Funahashi & Tanaka, 2001; Vozarova, Metz & Stefan, 2002). Unfortunately, with the exception of surgery (a procedure appropriate for only a minority of obese individuals), available treatments for obesity are, at best, of modest efficacy. With regard to nonsurgical treatments for obesity (Ayyad & Andersen, 1999), in a quantitative synthesis of the literature, found that even in the best of conditions, the median percentage of patients who

achieved and maintained clinically meaningful weight loss for at least 3– 5 years was only about 20%, whereas others have reported that of those who lose weight, 90–95% eventually regain it (World Health Organization, 2000).

Pre-clinical evaluatory study of compound can be done by using three animal models i.e. drug induced obesity; food induced obesity and genetically modified C57BL/6J female mice. Theneuro active steroid, progesterone is a female reproductive hormone. Its level increases during the later phase of the menstrual cycle and controls the secretary phase of the endometrium. Substantial evidence links progesterone excess in pathophysiology of eating and affective disorders. Some reports suggest the use of progesterone containing preparations as contraceptive or for the hormone replacement therapy to cause sufficient weight gain by causing hyperphagia and increased fat deposition in the body (Amatayakul, Sivasomboon & Thanangkul, 1980; Wallace, Shively & Clarkson, 1999). Reports also suggest that progesterone can produce these effects by inducing myriad of neurotransmitter changes of which alterations of serotonin level can be important. With this setting we have chosen this neuroactive to induce obesity in female mice. It is well known that high fat intake and sedentary life style, white collar jobs, lack of exercise etc. causes fat accumulation an increase in body weight. Cafeteria diets animal models of obesity have been reported to bear close resemblance to human obesity (Sclafani & Springer, 1976). Cafeteria diet is the combination of different composition like supermarket highly palatable food. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as animals (Bull 1988; Rothwell, Stock & Warwick, 1983). In general, C57BL/6J (*ob/ob*) models have been used for investigation of human obesity and metabolic syndrome (Feldstein, *et. al.*, 2003). When raised on a low-fat diet, the C57BL/6J *(ob/ob)* mice are lean and euglycemic with normal insulin levels and blood pressure. However, when raised on a high-fat (HF) diet, animals develop central adiposity, hyperinsulinemia, hyperglycemia and hypertension (Surwit, Kuhn, Cochrane, McCubbin & Feinglos, 1988). These syndromes appear to be related to abnormalities in adrenergic control of adipocyte function, which, in turn, appear to be related to hyperinsulinemia (Surwit, Kuhn, Cochrane, McCubbin, & Feinglos, 1988). Also, the development of insulin resistance, hyperglycemia and obesity in the

C57BL/6J (*ob/ob*) mice closely parallel the progression of common forms of the human diseases (Crevel, Friend, Goodwin & Parish, 1992).

Ayurvedic system of medicine is one of the oldest system of medicine having a history of more than 3000 years. Several prototype derived from these herbal medicines are in use for various kind of disease and disorders. It not only gives new molecule but also with newer mechanism of action, hence is called Gold mine. Several infusions or decoctions of plants used in traditional medicine to reduce obesity could be utilized to delete the clinical side effects of the current chemically formulated antiobesity agents; examples include *Camellia sinensis* (L.) Kuntze (Theaceae), *Chlorella pyrenoidosa* Chick. (Oocystaceae), *Citrus aurantium* L. (Rutaceae), *Garcinia cambogia* L. (Clusiaceae), *Lagerstroemia speciosa* (L.) Pers. (Lythraceaea), *Panax ginseng* C.A. Meyer (Araliaceae), *Salix matsudana* Koidzumi (Salicaceae), *Nelumbo nucifera* Gaertn. (Nymphaeaceae) and *Ste llaria media* and *Clerodendrum phlomites* (Calapai, *et, al.,* 1999; Han *et. al.*, 2003; Hidaka, Okamoto & Arita, 2004; Dilip, Manashi & Nazim, 2005; Katewa & Galav, 2006). A large study of literature indicates that substantial progress has been made concerning our knowledge of bioactive components in plant foods and their links to obesity. For the present research protocol we have chosen *Clerodendrum serratum linn.* to evaluate its anti-obesity.As per the literature survey it was found that flavonides, sitosterols, tannins and saponines have shown the anti-obesity activity by various mechanisms, this plant have shown the presence of some common phytoconstituents in their extracts like sitosterols, triterpenoids, flavonoids and tanins etc. Moreover traditional system of Indian medicine also claims for its anti-obesity activity. With this back ground we have selected this plant for its phytochemical analysis, screening its anti-obesity to substantiate the folklore claim.

1.2. Disease profile:

1.2.1. Definition:

Obesity is a chronic disease that causes risks ill health, impaired quality of life, and premature mortality. Obesity results from a complex interaction of genetic predisposition, environmental, societal and individual psychological factors that all

summate to produce a chronic positive energy balance (Finer, 2002).Obesity results from a positive energy balance i.e., when caloric intake chronically exceeds energy expenditure. This in turn causes excess of adipose tissue mass with body mass index (BMI) > 30 kg/m^2) (Caterson, 1999).

The classification of body weight according to WHO is as follows:

BMI < 18.5	Underweight
BMI 18.5 – 24.9	Normal
BMI 25 - 29.9	Over weight
BMI 30 – 39.9	Obese
BMI 40 or higher	Severely obese

Although there is a correlation between obesity and cardiac disease, BMI is not a precise predictor of cardiovascular disease; absolute waist circumference or waist to hip ratio are more precise measures of central obesity and correlate better to health risks than BMI (Janssen, Katzmarzyk & Ross, 2004).

According to the American Cancer Society, obesity cost an estimated $75 billion in 2003 because of the long and expensive treatment for several of its complications. According to the National Institute of Health**,** $75 -$125 billion is spent on indirect and direct costs due to obesity-related diseases.(http://www.phitamerica.org/Cost_of_Obesity_Counter.htm)

Eric Schlosser in his book "Fast Food Nation" states that the annual health care costs in the United States stemming from obesity approaches $240 billion.

1.2.2. Obesity and insulin resistance diabetes

Obesity-associated insulin resistance is a major risk factor for type 2 diabetes and cardiovascular disease. In the past decade, a large number of endocrine, inflammatory, neural, and cell-intrinsic pathways have been shown to be

dysregulated in obesity. Although it is possible that one of these factors plays a dominant role, many of these factors are interdependent, and it is likely that their dynamic interplay underlies the pathophysiology of insulin resistance. Understanding the biology of these systems will inform the search for interventions that specifically prevent or treat insulin resistance and its associated pathologies. (Mohammed Qatanani & Mitchell A. Lazar, 2007).

One of the most common physical consequences of obesity is the development of insulin resistance. Insulin resistance is an irreversible and progressive condition; once the body's cells do not respond to insulin, the glucose levels must be controlled through human intervention and monitoring. In obese persons, insulin resistance is another stressor on the body that heightens the considerable risk of early mortality. Insulin resistance has other serious consequences, as it will often lead to the progressive illness known as the metabolic syndrome, which is the combination of a series of distinct physical conditions. Each component of the metabolic syndrome is potentially dangerous to human health when present alone: type 2 diabetes mellitus, hypertension, hyperlipidimedia (the production of excessive levels of low density lipoproteins, a harmful cholesterol that causes plaque to form inside the blood vessels, which causes a narrowing and the potential for restricted blood flow), cardiovascular disease, renal failure, risk of various infections, particularly when the skin is cut or a sore develops.

1.3. Epidemiology

Globally, more than 1.1 billion adults worldwide are overweight, and 312 million of them are obese. In addition, at least 155 million children worldwide are overweight or obese, according to the International Obesity Task Force (Childhood Report). This task force and the World Health Organization (WHO) have revised the definition of obesity to adjust for ethnic differences, and this broader definition may reflect an even higher prevalence with 1.7 billion people classified as overweight worldwide (N Engl 2007).

About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors. Propelling the

upsurge in cases of diabetes and hypertension is the growing prevalence of overweight and obesity (Report of the International Bioethics Committee of UNESCO (IBC) on Social Responsibility and Health).

Data from the National Health and Nutrition Examination Surveys (NHANES) show that the percent of the American adult population with obesity (BMI > 30) has increased from 14.5% (between 1976 and 1980) to 30.5% (between 1999 and 2000). As many as 64% of U.S. adults = 20 years of age were overweight (defined as BMI > 25) between the years of 1999 and 2000. Extreme obesity (BMI = 40) has also increased and affects 4.7% of the population. The increasing prevalence of medically significant obesity raises great concern. Obesity is more common among women and in the poor; the prevalence in children is also rising at a worrisome rate (Kasper, *et. al.*, 2005).

In the past 20 years, the rates of obesity have tripled in developing countries that have been adopting a Western lifestyle involving decreased physical activity and overconsumption of cheap, energy-dense food. Such lifestyle changes are also affecting children in these countries; the prevalence of overweight among them ranges from 10 to 25% and the prevalence of obesity ranges from 2 to 10%. The Middle East, Pacific Islands, Southeast Asia and China face the greatest threat. In India currently, almost 1 in 5 men and over 1 in 6 women are overweight. In some urban areas, the rates are as high as 40%. Published in the Lancet the Organization for Economic Co- operation and Development (OECD), the study warns that low-income countries cannot cope with the health consequences of wide scale obesity (Kounteya, 2010).

The growing prevalence of type 2 diabetes, cardiovascular disease,and some cancers is tied to excess weight. The burden of these diseases is particularly high in the middle-income countries of Eastern Europe, Latin America, and Asia, where obesity is the fifth-most-common cause of the disease burden ranking just below underweight. The high risk of both diabetes and cardiovascular disease associated with obesity in Asians may be due to a predisposition to abdominal obesity, which can lead to the metabolic syndrome and impaired glucose tolerance. The serious cardiovascular complications of obesity and diabetes could overwhelm developing

countries that are already straining under the burden of communicable diseases. The increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity. About 90% of type 2 diabetes is attributable to excess weight. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome. This number is expected to increase to 420 million by 2025.

Cardiovascular disease is considerably greater among obese people, and this group has an incidence of hypertension that is five times the incidence among people of normal weight. Hence, overweight and obesity are contributing to a global increase in hypertension. 1 billion people had hypertension in 2000 and 1.56 billion people are expected to have this condition by 2025 (Kearney, Whelton, Reynolds, Muntner & Whelton, 2005).

Obesity, diabetes, and hypertension also affect the kidneys. Diabetic nephropathy develops in about one third of patients with diabetes and its incidence is sharply increasing in the developing world, with the Asia–Pacific region being the most severely affected. According to a survey published in 2003, diabetic nephropathy was the most common cause of end-stage renal disease in 9 of 10 Asian countries, with an incidence that had increased from 1.2% of the overall population with end-stage renal disease in 1998 to 14.1% in 2000 (Parvez hossain,et.al., 2007).

1.4. Etiology of obesity

The exact etiology of obesity is unclear. The multiple causative factors like genetic, environmental, nutritional, physiological, psychological, social and cultural factors have been linked to its development and progression (Rippe, Crossley& Ringer, 1998).

Though the molecular pathways regulating energy balance are beginning to be illuminated, the causes of obesity remain elusive. In part, this reflects the fact that obesity is a heterogeneous group of disorders. At one level, the pathophysiology of obesity seems simple: a chronic excess of nutrient intake relative to the level of energy expenditure. However, due to the complexity of the neuroendocrine and metabolic systems that regulate energy intake, storage, and expenditure, it has been

difficult to quantitate all the relevant parameters (e.g., food intake and energy expenditure).

1.4.1. Environmental factors

The current environmental risk factors include over consumption of energy (increase in fat to carbohydrate ratio) and decrease in physical activity. These factors offer more reasonable explanation for the recent dramatic surge in the prevalence of obesity (Poppitt, 1995).

1.4.2. Nutritional factors

Numerous metabolic studies have shown that high fat diets may lead to a high energy intake and hyperphagia. The reason may be that fat has a weaker effect on the satiety centre and on heat production (diet-induced thermogenesis) and it possesses a higher energy density compared to carbohydrates. Also fats are highly palatable and heighten the flavour of food stuffs which leads to their passive overconsumption. This ultimately increases fat deposits and causes obesity and related problems (Zhang, *et. al.,* 1994).

1.4.3. Physiological factors

These involve the impairment of the central mechanism regulating appetite and food intake which is thought to be regulated by a complex interplay of neurotransmitters in the hypothalamic region of the brain. Approximately 1 - 2% of obesity can be ascribed to lesions in hypothalamic regulatory centres. Such lesions may be due to trauma, tumours, inflammatory processes, or carotid artery aneurysms (Bray & York, 1979).

1.4.4. Psychological factors

The psychogenic theory of obesity long held that obesity resulted from an emotional disorder in which food intake, relieved the anxiety and depression to which obese persons are usually susceptible. Stress associated with traumatic emotional events has been held responsible for certain cases of obesity and has been

implicated in the pathogenesis of eating disorders such as night-eating syndrome and bulimia (Kissebah *et. al.*, 1982).

1.5. Role of Genes versus Environment

Obesity is commonly seen in families, and the hereditability of body weight is similar to that for height. Inheritance is usually not Mendelian, however, and it is difficult to distinguish the role of genes and environmental factors. Adoptees usually resemble their biologic rather than adoptive parents with respect to obesity, providing strong support for genetic influences. Likewise, identical twins have very similar BMIs (Body Mass Index) whether reared together or apart, and their BMIs are much more strongly correlated than those of dizygotic twins. These genetic effects appear to relate to both energy intake and expenditure.

Whatever the role of genes, it is clear that the environment plays a key role in obesity, as evidenced by the fact that famine prevents obesity in even the most obesity-prone individual. In addition, the recent increase in the prevalence of obesity in the United States is too rapid to be due to changes in the gene pool. Undoubtedly, genes influence the susceptibility to obesity when confronted with specific diets and availability of nutrition. Cultural factors are also important these relate to both viabilityand composition of the diet and to changes in the level of physical activity. In industrial societies, obesity is more common among poor women, whereas in underdeveloped countries, wealthier women are more often obese. In children, obesity correlates to some degree with time spent watching television. High fat diets may promote obesity, as may diets rich in simple (as opposed to complex) carbohydrates (Kasper *et. al.*, 2005).

1.6. Specific Genetic Syndromes

For many years obesity in rodents has been known to be caused by a number of distinct mutations distributed through the genome. Most of these single-gene mutations cause both hyperphagia and diminished energy expenditure, suggesting a link between these two parameters of energy homeostasis. Identification of the *ob* gene mutation in genetically obese *(ob/ob)* mice represented a major breakthrough in the field. The *ob/ob* mouse develops severe obesity, insulin resistance, and

hyperphagia, as well as efficient metabolism (e.g., it gets fat even when given the same number of calories as lean littermates). The product of the *ob* gene is the peptide leptin, a name derived from the Greek root *leptos*, meaning thin. Leptin is secreted by adipose cells and acts primarily through the hypothalamus. Its level of production provides an index of adipose energy stores (Fig 1). High leptin levels decrease food intake and increase energy expenditure. Another mouse mutant, *db/db*, which is resistant to leptin, has a mutation in the leptin receptor and develops a similar syndrome. The *OB* gene is present in humans and expressed in fat. Several families with morbid, early-onset obesity caused by inactivating mutations in either leptin or the leptin receptor have been described, thus demonstrating the biologic relevance of leptin in humans. The obesity in these individuals begins shortly after birth, is severe, and is accompanied by neuroendocrine abnormalities. The most prominent of these is hypogonadotropic hypogonadism, which is reversed by leptin replacement. Central hypothyroidism and growth retardation are seen in the mouse model, but their occurrence in leptin-deficient humans is less clear. To date, there is no evidence to suggest that mutations or polymorphisms in the leptin or leptin receptor genes play a prominent role in common forms of obesity. Mutations in several other genes cause severe obesity in humans; each of these syndromes is rare. Mutations in the gene encoding proopiomelanocortin (POMC) cause severe obesity through failure to synthesize a-MSH, a key neuropeptide that inhibits appetite in the hypothalamus. The absence of POMC also causes secondary adrenal insufficiency due to absence of adrenocorticotropic hormone (ACTH), as well as pale skin and red hair due to absence of MSH. Proenzyme convertase 1 (PC-1) mutations are thought to cause obesity by preventing synthesis of a-MSH from its precursor peptide, POMC. a- MSH binds to the type 4 melanocortin receptor (MC4R), a key hypothalamic receptor that inhibits eating. Heterozygous mutations of this receptor appear to account for as much as 5% of severe obesity. These five genetic defects define a pathway through which leptin (by stimulating POMC and increasing MSH) restricts food intake and limits weight.

In addition to these human obesity genes, studies in rodents reveal several other molecular candidates for hypothalamic mediators of human obesity or leanness. The *tub* gene encodes a hypothalamic peptide of unknown function;

mutation of this gene causes late-onset obesity. The *fat* gene encodes carboxypeptidase E, a peptide- processing enzyme; mutation of this gene is thought to cause obesity by disrupting production of one or more neuropeptides. AgRP is coexpressed with NPY in arcuate nucleus neurons. AgRP antagonizes a- MSH action at MC4 receptors, and its overexpression induces obesity. In contrast, a mouse deficient in the peptide MCH, whose administration causes feeding, is lean. A number of complex human syndromes with defined inheritance are associated with obesity. Although specific genes are undefined at present, their identification will likely enhance our understanding of more common forms of human obesity. In the Prader- Willi syndrome, obesity coexists with short stature, mental retardation, hypogonadotropic hypogonadism, hypotonia, small hands and feet, fish-shaped mouth, and hyperphagia. Most patients have a chromosome 15 deletion. Laurence-Moon-Biedl syndrome is characterized by obesity, mental retardation, retinitis pigmentosa, polydactyly, and hypogonadotropic hypogonadism (Kasper, et. al., 2005).

1.7. Other Specific Syndromes Associated with Obesity:

InsulinomaPatients with insulinoma often gain weight as a result of overeating to avoid hypoglycemia symptoms. The increased substrate plus high insulin levels promote energy storage in fat. This can be marked in some individuals but is modest in most (Kasper, *et. al.,* 2005).

1.7.1. Cushing's syndrome

Although obese patients commonly have central obesity, hypertension, and glucose intolerance, they lack other specific stigmata of Cushing's syndrome. Nonetheless, a potential diagnosis of Cushing's syndrome is often entertained. Cortisol production and urinary metabolites (17OH steroids) may be increased in simple obesity. Unlike in Cushing's syndrome, however, cortisol levels in blood and urine in the basal state and in response to corticotropin-releasing hormone (CRH) or ACTH are normal; the overnight 1-mg dexamethasone suppressiontest is normal in 90%, with the remainder being normal on a standard 2-day low-dose dexamethasone suppression test. Obesity may be associated with local reactivation of cortisol in fat

by 11 ß hydroxysteroid dehydrogenase 1, an enzyme that converts cortisone to cortisol (Kasper *et. al*., 2005).

1.7.2. Hypothyroidism

The possibility of hypothyroidism should be considered, but it is an uncommon cause of obesity; hypothyroidism is easily ruled out by measuring thyroid-stimulating hormone (TSH). Much of the weight gain that occurs in hypothyroidism is due to myxedema (Kasper *et. al*., 2005).

1.7.3. Craniopharyngioma and other disorders involving the hypothalamus

Whether through tumors, trauma, or inflammation, hypothalamic dysfunction of systems controlling satiety, hunger, and energy expenditure can cause varying degrees of obesity. It is uncommon to identify a discrete anatomic basis for these disorders. Subtle hypothalamic dysfunction is probably a more common cause of obesity than can be documented using currently available imaging techniques. Growth hormone (GH), which exerts lipolytic activity, is diminished in obesity and is increased with weight loss. Despite low GH levels, insulin-like growth factor (IGF) I (somatomedin) production is normal, suggesting that GH suppression is a compensatory response to increased nutritional supply (Kasper *et. al.*, 2005).

1.8. TYPES OF OBESITY

1.8.1. Central (android) (apple) versus Peripheral (gynoid) (pear)

It has been noted that central (android, more prevalent in men) obesity is associated with a higher mortality than peripheral (gynoid, more prevalent in women) obesity. This discrepancy has been attributed to the fact that visceral adipose tissue is metabolically more active than subcutaneous fat, causing a greater rate of glucose production, type II diabetes mellitus, and hyperinsulinism. Increased insulin secretion is thought to increase sodium reabsorption and result in hypertension. Central obesity is also associated with increased production of cholesterol, primarily in the form of low-density lipoprotein, leading to increased

incidence of atherosclerotic cardiovascular disease and gallstones. The increased visceral fat has been related to an increased waist: hip ratio or, in more common terms, as the —apple versus —pear distribution of fat **(Figure 1)**. Computed tomography (CT) scans, however, have noted a much better correlation between anterior-posterior abdominal diameter and visceral fat distribution than the waist: hip ratio, especially in women with both central and peripheral obesity. In this situation the peripheral obesity —dilutes the central obesity as measured by the waist: hip ratio, so either waist circumference alone or sagittal abdominal diameter should be used as a measurement of central obesity.

A recent study documented increased bladder pressure in morbidly obese women that was associated with a high incidence of urinary incontinence. It is quite probable that much of the comorbidity of severe obesity is related to increased intra-abdominal pressure secondary to a central fat distribution and that urinary bladder pressure, surrogate for intra-abdominal pressure, is highly correlated with sagittal abdominal diameter or waist circumference (Sugerman, 2005). With analysis of surface area and volume, analysis of subcutaneous fat and intra-abdominal visceral fat could be done and a novel classification of obesity was proposed - visceral fat obesity (VFO) and subcutaneous fat obesity (SFO) based on the ratio of visceral fat area and subcutaneous fat area (V/S ratio) at the level of umbilicus (Yamashita, *et. al.* 1996; Arner, 1998).
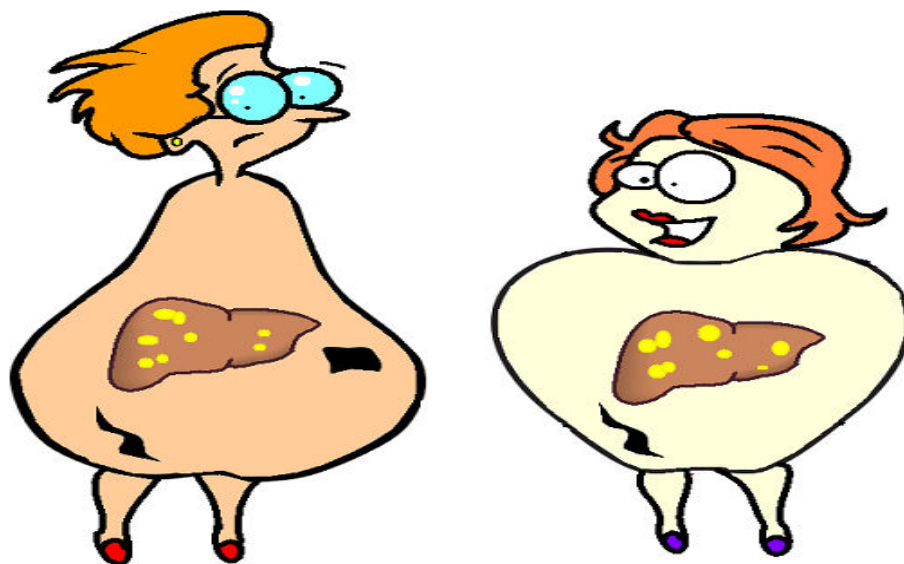
Figure 1: Left figure indicating the apple shape obesity where the fat mainly deposited above the waist where as right figure indicating pear shaped obesity where fat is mainly deposited below the waist.

1.8.2. Complications of obesity

Direct association between obesity and several diseases cause number of problems i.e., difficulties with daily activities to serious health issues.

Obesity Comorbidity: "the top of the head to the tip of the toes and almost every organ in-between". (Sugerman, 2005)

Head:

Brain Depression Stroke Headaches Hypertension Pseudotumor cerebri: Headaches

I. Optic nerve: Visusal field (Blindness)

II. Oculomotor nerve palsy

III. Trigeminal nerve: tic doloreaux

IV. Facial nerve: Bell's palsy

V. Auditory nerve: pulsatile tinnitus

Eyes:

Diabetic retinopathy

Mouth/throat Sleep apnea

Chest:

Breast cancer

Obesity hypoventilation

Heavy chest wall

Elevated diaphragm

Increased Intrathoracic pressure

Decreased expiratory reserve volume

Heart:

Left ventricular hypertrophy

Eccentric: Increased cardiac output

Concentric: increased peripheral vascular resistance

Increased cardiac filling pressures (CVP, PAP, WP)

Right heart failure

Tricuspid insufficiency

Esophagus:

Acid reflux

Asthma

Adenocarcinoma

Esophageal varices

Abdomen:

Gallbladder Cholecystitis

Adenocarcinoma

Liver:

Non-alcholic liver disease (NALD)

Non-alcoholic steatohepatitis (NASH)

Cirrhosis Type 2 diabetes mellitus

Spleen:

Splenomegaly (portal hypertension)

Hypersplenism (portal hypertension)

Pancreas:

Type 2 diabetes mellitus

Necrotizing pancreatitis

Colon

Adenocarcinoma

Diverticulitis

General:

Difficulty diagnosing peritonitis

Hernia

Incsional Inguinal Spighelian

Wound infection

Lymphatic stasis

Kidney:

Hypertension

Proteinuria

Renal cell carcinoma

Urinary bladder Stress incontinence

Ovaries/uterus:

Increased estradiol,

androstenedione

Polycystic ovary syndrome,

Stein–Leventhal syndrome

Infertility

Dysmenorrhea

Hirsutism

Endometrial carcinoma

Breast cancer

Pregnancy complications

Maternal, fetal mortality

Pre-eclampsia,

Eclampsia

Gestational diabetes

Thromboembolism

Complicated childbirth: cephalopelvic disproportion, increased cesarean section fetal complications: macrosomia, shoulder dystocia, small for gestational age

Prostate: adenocarcinoma

Anus:

Periananal abscess

Necrotzing panniculitis

Integument:

Necrotizing panniculitis

Hirsutism

Increased risk of operative complications

Colectomy

Hysterectomy

Kidney, liver transplantation

Spine:

Herniated disc

Upper Extremties:

Shoulder girdle pain Edema

Lower extremities:

Osteoarthritis

Hip arthralgia

Knee arthralgia

Venous stasis:

Edema

Thrombophlebitis

Stasis ulcers

Pulmonary embolism

Lymphedema

Toes:

Diabetic neuropathy Diabetic ulcers

1.9. The adipocyte and adipose tissue

Adipose tissue is composed of the lipid-storing adipose cell and a stromal/vascular compartment in which preadipocytes reside. Adipose mass increases by enlargement of adipose cells through lipid deposition, as well as by an increase in the number of adipocytes. The process by which adipose cells are derived from a mesenchymal preadipocyte involves an orchestrated series of differentiation steps mediated by a cascade of specific transcription factors. One of the key transcription factors is *peroxisome proliferatoractivated receptor □ (PPAR□)*, a nuclear receptor that binds the thiazoladinedione class of insulin-sensitizing drugs used in the treatment of type 2 diabetes.

Although the adipocyte has generally been regarded as a storage depot for fat, it is also an endocrine cell that releases numerous molecules in a regulated fashion. These include the energy balance-regulating hormone leptin, cytokines such as tumor necrosis factor (TNF) □, complement factors such as factor D (also known as *adipsin*), prothrombotic agents such as plasminogen activator inhibitor I, and a component of the blood pressure regulating system, angiotensinogen. Adiponectin (or ACRP30) enhances insulin sensitivity andlipid oxidation, whereas resistin may induce insulin resistance. These factors, and others not yet identified, play a role in the physiology of lipid homeostasis, insulin sensitivity, blood pressure control, and coagulation and are likely to contribute to obesity-related pathologies.

1.10. Management of obesity

Currently three options are available for the treatment of obesity and associated complications are non pharmacological treatment, pharmacotheropy and surgical procedures, each of these are having their own advantageous and drawbacks.

1.10.1 Non Pharmacological approach

Non-pharmacological measures are preferred for many reasons for many reasons including adverse effects of anti-obesity drugs, contraindications or allergic reactions to drugs, perceptions of adverse effects of drugs, or personal preference for natural or alternative therapies. A more aggressive integrative approach to the management of obesity is recommended to improve outcomes, minimize adverse effects, and reduce health care costs. Non-pharmacological treatment consists of lifestyle modification, reduction of total caloric intake and regular aerobic exercise.

Diet:

There is no evidence to suggest that specific components of the diet (ie, carbohydrate, fat, protein, vitamins, micronutrients) influence the ways in which food energy is absorbed or used. Therefore, the main dietary approach for reducing weight is to reduce the total amount of calories consumed, and this is best achieved by a reduction in the amount of fat in the diet and calories from soft drinks. A moderate decrease in caloric balance (500-1000 kcal/d) will result in a slow but progressive weight loss (Franz, *et. al.*, 2002). In addition, evidence suggests that the components of diet currently recommended as healthy, including low consumption of saturated and trans fats, intake of carbohydrates that are rich in dietary fiber, high fruit and vegetable intake, and the inclusion of low-fat dairy foods - are likely to protect against metabolic syndrome (Feldeisen & Tucker, 2007). However, even if dietary efforts are the primary treatment approach for people who are overweight or obese, dietary counseling interventions generally produce only modest weight loss that diminishes over time, as emphasized in a recent meta-analysis (Dansinger *et. al.*, 2007).

Exercise:

Most obesity studies have not adequately measured physical activity and functional capacity, and the independent contributions of "fitness" versus "fatness" to health risks associated with obesity are still being debated (Blairi & Church, 2004). Nonetheless, the role of physical activity as a treatment and/or preventive strategy for combating obesity has been the subject of substantial research. A systematic review of the literature concluded that limited evidence from a number of studies that used imaging techniques to quantify changes in abdominal obesity suggests a beneficial influence of physical activity on reduction of abdominal fat and VAT in overweight and obese subjects (Kay & Fiatarone, 2006). Reductions in VAT and total abdominal fat may occur in the absence of changes in body mass and waist circumference. Because the deposition of fat in the abdomen and in non-adipose tissues such as liver (Yki-Jiirvinen, 2005) and muscle (Moro, Bajpeyi & Smith, 2008) plays a major role in the development of obesity-related health risks, these depots have emerged as alternative targets for obesity treatment and may partly explain the utility of physical activity with only minimal or no weight loss in the treatment of obesity (Janiszewsk & Ross, 2007). However, more rigorous studies are needed to confirm these observations.

Lifestyle Modifications:

The primary approach for achieving weight loss, in the vast majority of cases, is lifestyle modification, including a reduction in energy intake and an increase in physical activity (Scheen, 2008; Scheen, 2004).

1.10.2. Pharmacological measures of obesity:

Obesity therapies include reducing nutrient absorption and applying anorectic drugs, thermogenic drugs or drugs that affect lipid mobilization and utilization. With the exception of Orlistat, a recently approved gastrointestinal lipase inhibitor, all drugs approved for the treatment of obesity are either catecholaminergic or serotonergic CNS-active (activating the sympathetic nervous system) anorectic agents. Since some of these drugs may lead to dependency, they

are recommended for short-term use like amphetamine-like drugs. Upon termination of therapy with these drugs, weight is rapidly regained in many cases (Kang, *et. al.*, 2004).

Dinitrophenol was the first synthetic thermogenic drug used to treat obesity, unfortunately during clinical use there were number of deaths from multisystem side- effects and the drug was rapidly withdrawn. The discovery of ephedrine led to synthesis of amphetamines which suppressed appetite and food intake, but stimulation actions of these drugs produced strong liability to abuse (Finer, 2002).

Currently used anti-obesity drugs include inhibitors of 5-hydroxytryptamine uptake like fenfluramine and sympathomimetics like phentermine. Fenfluremine is reported to cause pulmonary hypertension and heart valve defects whereas phentermine may produce rebound weight gain. ALT-962 is a new molecule as the sole competitor of orlistat, which is found to be safe and well tolerated in phase I of clinical trials, is now being forwarded for phase II and III studies (Goyal & Shah, 2002).

Concern of risk factors associated with anti-obesity drugs the British National Formulary recommends thatdrugs (for obesity) should only be considered for those with a BMI of 30 or greater if supervised diet, exercise and behaviour modification fail to achieve a realistic reduction in weight(Greenway, 1996).

Surgery can provide palliation for severe obesity when all medical approaches have failed. It can result in decreased food intake (gastric procedures - jaw wiring, vertical banded gastroplasty, gastric stapling), affect calorie absorption (intestinal shunting, biliopancreatic bypass), or remove excess fat (lipectomy, liposuction) (Pasquali & Casimirri, 1993).

1.10.3. Surgical Approach:

The contrasting effects of various surgical procedures on the metabolic profile have underscored the crucial role of intra-abdominal adipose tissue (ie, VAT) rather than subcutaneous abdominal adipose tissue **(Figure 2)**. Indeed, whereas large volume liposuction that reduces abdominal subcutaneous fat depots by 8 to 10 kg

has almost no favorable effects on the metabolic profile, Omental fat reduction (corresponding to only 0.8% of total body fat) in connection with adjustable gastric banding results in a dramatic improvement in insulin resistance and associated glucose disturbances (Klein, *et. al.*, 2004; Thörne, Lönnqvist, Apelman, Hellers, & Arner, 2002). Bariatric surgical procedures (i.e. gastroplasty, gastric bypass) are the only procedures that provide marked and sustained weight reduction in morbidly obese patients, leading to improvements in associated metabolic disorders, especially type 2 DM, and a more favorable long-term prognosis, including a reduction in total mortality (Sjöström, *et. al.*, 2007). However, considering the risk/benefit ratio of bariatric surgery, it may not yet be considered an early option in the management of the abdominally obese patient.
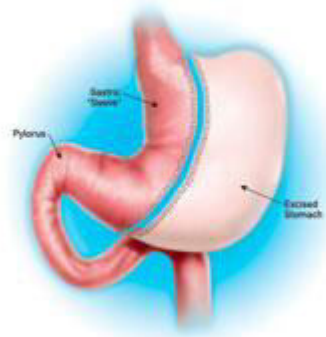
Figure 2. Surgical method (Surgical Gastric sleeve Method)

1.11. Herbal plants against Obesity

Medicinal plants and plant extracts represent the oldest and most widespread form of medication. At least 25% of the active compounds in currently prescribed synthetic drugs were first identified in plant sources (Balandrin, Klocke, Wurtele & Bollinger, 1985).

Dissatisfaction with the high costs and potentially hazardous side effects of pharmaceuticals have resulted in a larger percentage of people in the United States purchasing and exploring the applications of medicinal plants than before (Kessler *et. al.*, 2001). Several plants like willow, poppy, foxglove, cinchona, aloe and garlic have been verified as medicinally beneficial through repeated clinical testing and

laboratory analyses (Youngkin, & Israel, 1996; O'Hara, Kiefer, Farrell, & Kemper, 1998) and a number of plant extracts like green tea (Hasegawa, Yamda, & Mori, 2003), garlic compounds (Elkayam, *et. al.*, 2003) and conjugated linoleic acid (CLA)(Hargrave, *et. al.*, 2002) were shown to possess either antidiabetic effects or have direct effects on adipose tissue. A large body of literature indicates that substantial progress has been made concerning our knowledge of bioactive components in plant foods and their links to obesity. Polyphenols constitute one of the ubiquitous groups of plant metabolites (Bravo, 1998) widely found in fruits, vegetables, cereals, legumes and wine (Aherne, O'Brien, 2002; Harborne, 1989).

A number of studies have been carried out to investigate the antiobesity effects of polyphenols like apigenin and luteolin (Han, *et. al.*, 2003), kaempferol (Yu, *et. al.*, 2006), myricetin and quercetin (Kwon, *et. al.*, 2007),genistein and diadzein (Kim *et. al.*, 2006; Naaz *et. al.*, 2003; Dang & Lowik 2004),cyaniding (Tsuda, Ueno, Kojo, Yoshikawa & Osawa, 2005) grape seed proanthocyanidin extract (GSPE) (Preuss, *et. al.*, 2000), xanthohumol (Nakagawa *et. al.*, 2005) and epigallocatechin gallate (EGCG) (Wolfram *et. al.,* 2006). Likewise, studies involving the effects on lipid metabolism have been carried out with carotenoids like fucoxanthin (Maeda *et. al.,* 2005),coumarin derivatives like esculetin (Yang *et. al.*, 2006) and phytoalexins like resveratrol (Picard *et. al.*, 2004). Other bioactive components of food with antiobesity effects include phytosterols, polyunsaturated fatty acids and organosulfur compounds.

2. PLANT PROFILE

Fig-3. *DELONIX ELATA*

Table - 1 : Scientific Classification

Scientific Classification	
Kingdom	*Plantae*
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Leguminosae
Genus	Delonix
Species	*Elata*

Table 2

Vernacular names	
Tamil	Vadanarayani
Hindi	Sankasura,
Gujrathi	Sandesra
Sanskrit	Siddhesvara

Habitat:

Delonix elata has a widespread distribution and is found naturally between 25 degrees north to 8 degrees south. Native countries Djibouti, Egypt, Ethiopia, Kenya, Saudi Arabia, Somalia, Sudan, Tanzania, United Republic of, Uganda, and Yemen. Introduced in Cambodia, Maldives, Myanmar, Namibia, Nigeria, Oman, Pakistan, and Sri Lanka. Present, but origin uncertain in India and Zambia. Also widely cultivated as an avenue tree and for shade elsewhere in the tropics.

Description:

Delonix elata is a perennial, medium-sized, deciduous tree about 2.5-15 m tall, with a spreading, rather rounded crown and very attractive, white fragrant flowers. *Delonix elata* is a variable species showing correlation with geography, variations mostly regarding number of pinnae pairs per leaf, and density of pubescence in the inflorescence (including the outside of the calyces). There are, however, good reasons for not recognizing these local or morphological forms as subspecies or varieties. This tree is a close relative of popular *Delonix regia* and has similar growth habit and characteristics, but differs from the latter in having small subulate deciduous stipules; white, yellow or orange petals which are only 1,6-3,8 cm long; stamen-filaments which exceed the petals; and smaller pods, 13-26 cm long, 2,1-3,7 cm wide.

Chemical Constituents:

The plant bark posessess beta sitosterol,saponins, alkaloids, carotene.

Medicinal properties:

The leaf and bark extracts of D. elata are anti-inflammatory agents; a root decoction is drunk for abdominal pains. Leaves are reported to be used by traditional practitioners in cases of inflammatory joint disorders as a folklore remedy. A pychosomatic medicinal use relating to scorpion bite treatment is reported from India. Leaf and seed extracts have anti malarial and antiovicidal activity; hence these extracts are used by traditional practitioners to treat malaria.

LITERATURE REVIEW

The objective of this review article is to highlight all the available information online or offline in the form of books or articles on Delonix elata (L.), one of most important medicinal plants. Methods: This article is a compiled report on medicinal properties and phytochemistry of D. elata based on the updated information collected from reviews, literature databases, research articles and books. Results: Delonix elata (L.) Gamble (Poinciana elata Linn.) commonly known as "white gul mohur" in English and is a reputed folklore remedy for arthritic disorders in many parts of Gujarat, India. The leaf extracts are anti-inflammatory agents. Root decoction of this plant is consumed for abdominal pains. Leaves are reported to be used by traditional practitioners for inflammatory joint disorders as a folklore remedy. Its pychosomatic medicinal use relating to scorpion bite treatment is also reported. The presence of phytochemicals such as alkaloids, tannins, triterpenoids, steroids and glycosides in the extracts of this plant supports its traditional uses as a potent medicinal plant for the treatment of various ailments. Conclusion: Scientific investigations are needed to be carried out on D. elata to bring such unexplored drugs into light to combat with various human diseases.

Antioxidant activity:

Ethanolic extract of *Delonix elata* is reported for its free radical scavenging property on different *in vitro* models, *viz,* 1,1-dipheny12-picrylhydrazine (DPPH), hydrogen peroxide, total antioxidant capacity and peroxy radical model. The *in vitro* lipid peroxidation (LPO) is also reported to be inhibited to a good extent by theethanolic leaf extract of *Delonix elata* [14,15]. The medicinalproperties of plants have been investigated in the recent scientificdevelopments throughout the world, due to their potentialantioxidant activities, no side effects and economic viability.

Themajority of the active antioxidant compounds flavonoids,isoflavones, flavones, anthocyanins, coumarins, lignans, catechins,

and isocatechins. In addition to the above compounds found innatural foods, vitamins C and E, b-carotene, and a-tocopherol areknown to possess antioxidant potential [16-18]..

ANTI-INFLAMMATORY ACTIVITY OF DELONIX ELATA.

(Int J Pharm Pharm Sci, Vol 5, Suppl 4, 1-3).

Delonix elata is known to be used for joint pains and in flatulence. It was accidentally observed that local people of some regions using the leaves and bark of Delonix elata in inflammation. There was n report on anti-inflammatory activity of Delonix elata. Antiinflammatory activity of the alcoholic extracts of the leaves and bark of Delonix elata was found to be significant.

Anti-inflammatory activity of this plant is reported using carageenin induced oedema model [19-20]. The paw oedema was measured by using plethysmograph. The LD50 values of this plant extracts are reported as more than 100 mg/kg/b.w. in mice and the active principles in extracts are present usually in small quantities. A dose 300 mg/kg/b.w. was administered to assess the validity as known anti-inflammatory agent in comparison with phenylbutazone. The report indicates that the bark extract showed slight lower response than phenylbutazone (50 mg/kg). The leaf extract also showed significant anti-inflammatory action compared to control but it was lower than the effect of bark extract. Compounds like bioflavonoid are reported to produce anti-inflammatory action by decreasing capillary permeability [21]. Steroids are known to produce anti-inflammatory activity. The extracts tested might contain flavonoids/steriodis which resulted in producing antiinflammatory activity [22].

Antibacterial activity:-

The antibacterial activity of organic solvent extracts of this plant was determined by disc diffusion and broth dilution techniques against gram-positive bacterial strains (Bacillus subtilis, Staphylococcus aureus) and gram-negative bacterial strains (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa). The chloroform and methanol extracts exhibited significant antibacterial activity against gram-positive and gram-negative strains with minimum bactericidal concentration (MBC) ranging from 1.5 to 100 mg/ml. The presence of phytochemicals such as alkaloids, tannins, triterpenoids, steroids and glycosides in the extracts of these plants supports their traditional uses as medicinal plants for the treatment of various ailments. The observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts of plants tested. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms [23, 24] and offer protection to plants themselves against pathogenic microbial infections [25].

3. AIM & OBJECTIVE

The Goal of the present study is to investigate

- Anti- hyperlipidemic activity of aerial parts of *Delonix elata* on high cholesterol diet induced hyperlipidemia in rats.

The following objectives were set to achieve the goal of the present study

1. To perform the phytochemical evaluation of ethanolic extracts of aerial parts of *Delonix elata* [EEDE].
2. To perform the acute toxicity studies of ehanolic extracts of *Delonix elata* on mice.
3. To investigate the biochemical estimations of various doses of *Delonix elata* on lipid profile (TC, TG, HDL, LDL, VLDL) and kidney parameters urea, uric acid and creatinine in all the groups.
4. To investigate the effect of various doses of *Delonix elata* on antioxidant enzymes (SOD, GSH and catalase) in all the groups
5. Histopathology studies of heart.

4. PLAN OF WORK

I. PHYTOCHEMICAL SCREENING:

Collection:

Collection of *Delonix Elata. barks*, authentication and shade drying.

Extraction:

Extraction of powdered barks with 70% ethanol.

Preliminary Phytochemical examination for identification of chemical constituents.

II. PHARMACOLOGICAL SCREENING:

Acute oral toxicity study of ethanol extract of *Delonix Elata.* (OECD Guideline 423).

Evaluation of *Delonix Elata,*.extract on lipid profile, kidney parameters urea, uric acid and creatinine and on antioxidant enzymes (SOD, GSH and catalase) in all the groups.

Parameters Considered for evaluation.

Lipid Profile:

Serum Cholesterol

Triglycerides

High Density Lipoprotein

Low Density Lipoprotein

Very Low Density Lipoprotein

Kidney parameters:

Urea

Uric acid

Creatinine

Antioxidant enzymes:

Superoxide Dismutase (SOD)

Glutathione (GSH)

Catalase

Histopathological examination of Heart

Statistical Analysis:

4.2. Methods

Identification, collection and authentication of plant material:

The aerial parts of *Delonixelata* were collected from tirumala hills belong to Thirupathi, Andhra Pradesh, India. in the year of 2016 (May).

The plant specimen was authenticated by **Professor P. Jayaraman. Ph.D.,** founder of **Plant Anatomy Research Centre,** (PARC). Tambaram, Chennai. After cleaning parts of plant from foreign particles they spread over trays and separately dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve to get the fine powder, finally subjected to extraction.

Ethanolic extraction of aerial parts of *Delonixelata*

Introduction

The commonly employed technique for the separation of the active constituents from the crude drug is called extraction which involves the use of different solvents. Many of the complex substances metabolized by the plants have therapeutic importance. But these are always found in association with other substances. Therefore in order to study these active constituents alone it has to be separated from other unwanted substances produced.

Preparation of extract:

The aerial parts of *Delonixelata*were collected, washed, dried in shade and pulverized in a grinder- mixer to obtain a coarse powder and then passed through 40 mesh sieves. The powdered drug was subjected to solvent extraction by soxhlet apparatus.

Extraction procedure:

About 100g of powdered drug was extracted successively with 70% ethanol using soxhlet apparatus. The extraction was carried out for 72 hours until the extract becomes colourless. Then the solvent was completely removed by evaporating in rotatory flask evaporator. The dried extract thus obtained was kept in refrigerator until the further experiment.

Percentage yield:

❖ Percentage yield of 100gms of etahnolic extract of aerial parts of*Delonixelata*was found to be 14.6% w/w.

I. Preliminary photochemical screening:

The Ethanolic extract of aerial parts of *Delonixelata*(EEDE) was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as carbohydrates, alkaloids, saponins, phenolic compounds, gums, tannins and flavoniods.

The detailed study about the phytochemical test procedure as follows

A. Test for carbohydrates:

A small quantity of extracts was dissolved separately in distilled water and filtered. The filtrate was subjected to following tests

Molisch's test:

Take 2-3 ml of extract add 1ml of freshly prepared α-naphthol solution in alcohol shake and add conc.H_2SO_4 from sides of the test tube under tap water. Violet ring is formed at the junction of two liquids.

Benedict's test:

Take test solution and add benedicts reagent and heated on water bath and color was formed.

Fehling's test:

Take 1ml of extract add equal quantity of Fehling's solution A and B heat in boiling water bath for 5-10 minutes. Brick red precipitate is formed.

Barfoed's test:

Take 1ml of test solution add 1ml of Barfoed's reagent and heated on water bath. Red precipitate was formed.

B. Test for tannins

Colour reaction:

Tannins give color reactions with iron.

Catechol solution + Iron salts $\longrightarrow$ Green fluorescence

Condensed tannins + Iron salts $\longrightarrow$ Green fluorescence

Gallic acid + Iron salts $\longrightarrow$ Blue fluorescence

Ellagic acid + Iron salts $\longrightarrow$ Blue fluorescence

Matchstick test:

Dip matchstick in plant extract. Dry it. Moisten it with hydrochloric acid and warm near flame. Wood will turn pink or red in color due to phloroglucinol.

Gelatin test:

Solution of tannin (0.5% - 1%) precipitates 1% solution of gelatin containing 10% sodium chloride.

Phenazone test:

Take 5ml of aqueous extract of drug. Add 0.5 g of sodium acid phosphate. Warm it and cool, then filter solution. To the filtrate, add 2% solution of phenazone. Tannins will be precipitated. Precipitates will be bulky and colored.

Gold beater's skin test:

Soak a small piece of gold beater's skin in 2% hydrochloric acid. Rinse it will distilled water. Place it in solution to be tested for 5 minutes. Wash in water and transfer to 1% solution of ferrous sulphate. Black or brown color of skin indicates presence of tannins.

Catechin test:

Catechin when heated with acid produce phloroglucinol. Phloroglucinol can be detected with matchstick test. (Kokate et al.,2006 and khandelwal, 2006)

C. Test for alkaloids:

Dragendroff's test:

Add Dragendroff's reagent (potassium bismuth iodide solution) to the extract. Orange red precipitate is formed.

Mayer's test:

Take 1 ml of extract add 1ml of Mayer's reagent (potassium mercuric iodide). Cream coloured precipitate is formed.

Hager's test:

Take 1 ml of extract add 3 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow coloured precipitate indicates the presence of alkaloids.

Wager's test:

Take 1 ml of the extract add 2 ml of Wager's reagent (iodine in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

G. Test for indole alkaloids

Van-Urk's test:

Take test sample and add para-dimethyl amino benzaldehyde. Blue colour formed.

D. Test for saponins:

Foam test:

Take small quantity of ethanolic extract add 20 ml of distilled water, shaken in a graduated cylinder for 15 minutes, 1 cm layer of foam develops.

Hemolytic test:

Take drug extract or dry powder to one drop of blood, Placed on the glass side. Hemolytic zone appears.

D. Test for flavonoids:

Shinoda test:

Take ethanolic extract of powder add magnesium turnings or foil and add conc.Hcl. Intense cherry red colour or orange red colour is formed.

General tests:

To small quantity of residue, add lead acetate solution. Yellow color precipitate is formed.

a) Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration, which decolorizes after addition of acid.

E. Test for gums:

Mucilage test:

Take 10 ml of aqueous extract and add slowly to 25 ml of absolute alcohol with constant stirring. Filtered the precipitate and dried in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Hydrolytic test:

Hydrolyze the test solution using dilute Hcl. Perform Benedict's and Fehling's test. Red colour is formed.

F. Test for fixed oils and fats:

Spot test:

Press a small quantity of extract between the filter paper. Oil stains on the paper indicates fixed oils.

Saponification test:

Take 1 ml of the extract add few drops of 0.5N alcoholic KOH and drop of phenolphthalein. Heat the mixture on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali.

G. Test for acidic compounds:

To the extract add $NaHCO_3$ solution. Effervescence is observed.

To the extract add water and warm, filter. Litmus paper turns to blue colour

H. Test for amino acids:

Ninhydrin test:

Take small quantity of extract add 2 drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol). Heat for few minutes, blue colour is formed.

Biuret test:

Take 1 ml of 40%NaOH solution and 2 drops of 1% copper sulphate solution, add 1ml of extract. Blue colour is formed.

Xanthoproteic test:

Take test solution and add 1 ml of conc. nitric acid and boil. Yellow precipitate is formed. After cooling add 40% sodium hydroxide solution orange colour is formed.

Trytophan test:

Take 3 ml of test solution, a few drops of glyoxalic acid and conc.H_2SO_4. Reddish violet appears at the junction of two layers.

Cysteine test:

Take 5 ml of test solution add few drops of 40%NaOH and 10% lead acetate solution. Boil precipitate of lead sulphate is formed.

Millon's test:

Take 3 ml of test solution, mix with 5 ml of Millon's reagent. White ppt, warm ppt. turns brick red or the ppt. dissolves giving red colour solution, indicates the presence of tyrosine.

II. Acute toxicity studies

Experimental Animals:

Swiss albino mice (20-25gm) and Wistar albino rats (120-125gm) of male rats were purchased from Sri Venkateshwara Enterprises, Hyderabad, India. All animals were maintained in an air-conditioned room at 25°C±2°C, with a relative humidity of 75%±5%, and a 12-h light/dark cycle. A basal diet and tap water were provided ad libitum. Male and female rats were assigned to each dose group by stratified random sampling based on body weight. The animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments.

Experimental procedure:

Male albino mice weighing 20-25gm were used for the study. The starting dose level of EEDE . Baker was 5, 50, 300, 2000 and 5000 mg/kg body weight p.o. Dose was administered to overnight fasted mice's. Food was withheld for a further 3-4 hours after administration of *Delonixelata*and observed for signs for toxicity. The body weight of the mice's before and after administration were noted that changes in eyes and mucous membranes, skin and fur, respiratory, circulatory,

autonomic, and central nervous systems, and also motor activity and behavior pattern. Special attention was directed to observations of convulsions, tremors, diarrhea, salivation, lethargy, sleep, and coma were noted. The onset of toxicity and signs of toxicity of LD_{50} values are noted.

II. Pharmacological screening of hyperlipidemic activity:

Cholesterol
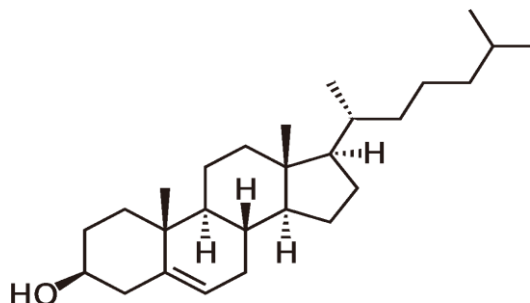
Cholesterol literally means "solid alcohol from bile". Cholesterol and triglycerides are two forms of lipid/fat. Cholesterol is made primarily in the liver (about 1,000 mg per day), but it is also created by cells lining the small intestine and by the individual cells in the body.

Cholesterol and other fats cannot dissolve in blood. They have to be transported to and from the cells by special carriers called "lipoproteins". Important lipoproteins are LDL and HDL.

The enzymes involved in the cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate to acetyl Co A provides all the carbon atoms in the cholesterol. Cholesterol synthesis takes place in 5 stages, which includes synthesis of HMG CO A, formation of mevalonate (6c), production of isoprenoid units (5c), synthesis of squalene (30c) and conversion of squalene to cholesterol (27c).

Cholesterol biosynthesis is controlled by the rate-limiting enzyme HMG CoA reductase, at the beginning of the pathway, which is found in association with endoplasmic reticulum and subjected to different metabolic controls like feedback control, hormonal regulation, inhibition by drugs and inhibition by bile acids.

Mechanism of action:

Biosynthesis of cholesterol is directly regulated by the cholesterol levels present, though the homeostatic mechanisms involved are only partly understood. A higher intake from food leads to a net decrease in endogenous production, whereas lower intake from food has the opposite effect. The main regulatory mechanism is the sensing of intracellular cholesterol in the endoplasmic reticulum by the protein SREBP (sterol regulatory element-binding protein 1 and 2). In the presence of cholesterol, SREBP is bound to two other proteins: SCAP (SREBP cleavage activating protein) and Insig1. When cholesterol levels fall, Insig-1 dissociates from the SREBP-SCAP complex, which allows the complex to migrate to the Golgi apparatus. Here SREBP is cleaved by S1P and S2P (site-1 and -2 protease), two enzymes that are activated by SCAP when cholesterol levels are low. The cleaved SREBP then migrates to the nucleus, and acts as a transcription factor to bind to the sterol regulatory element (SRE), which stimulates the transcription of many genes. Among these are the low-density lipoprotein(LDL) receptor and HMG-CoA reductase. The LDL receptor former scavenges circulating LDL from the bloodstream, whereas HMG-CoA reductase leads to an increase of endogenous production of cholesterol. Then, cholesterol can be filled in arteries which leads to plaque formation and causes insufficient supply of oxygen, B.P increases leads to coronary heart diseases. [American heart association]

Causes:

High cholesterol and other lipid disorders can be inherited (genetic) or associated with:

- Fatty diets
- Diabetes, hypothyroidism, Cushing's syndrome, and kidney failure
- Certain medications, including birth control pills, estrogen, corticosteroids, certain diuretics, and beta-blockers
- Lifestyle factors, including habitual, excessive alcohol use and lack of exercise, leading to obesity.

People who smoke and also have high cholesterol are at even greater risk for heart disease. Lipid disorders are more common in men than women.

Rosuvastatin:
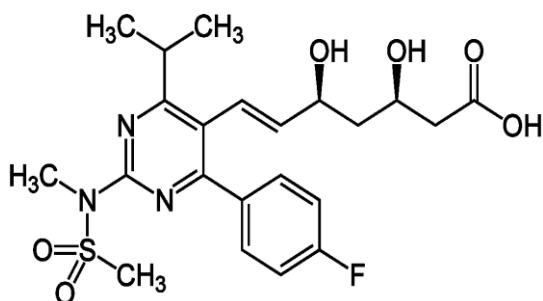
Systematic (IUPAC) name:-

(3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.

Rosuvastatin (marketed by AstraZeneca as **Crestor**) is a member of the drug class of statins, used in combination with exercise, diet, and weight-loss to treat high cholesterol and related conditions, and to prevent cardiovascular disease. The primary uses of rosuvastatin is for the treatment of dyslipidemia. It is recommended to be used only after other measures such as diet, exercise, and weight reduction have not improved cholesterol levels. Like all statins, rosvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body.

Mechanism of action:

Rosuvastatin inhibits cholesterol synthesis via the mevalonate pathway by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reductase is the enzyme responsible for the conversion of HMG-CoA to mevalonic acid, the rate-limiting step of cholesterol synthesis by this pathway. The active form of statins bears a chemical resemblance to the reduced HMG-CoA reaction intermediate that is formed during catalysis. Structure-activity relationship

studies have demonstrated that statins bind to HMG-CoA reductase at the same site as the reduced intermediate and are held in place by similar chemical interactions. Unlike Lovastatin and simvastatin, which undergo in vivo hydrolysis to their active form rosuvastatin is synthetically produced in active form. Cholesterol biosynthesis accounts for approximately 80% of cholesterol in the body; thus, inhibiting this process can significantly lower cholesterol levels [Reszka, A.A et al., 2003 and Schmidt, E.B et al., 2004].

Dyslipidemia:

Hypercholesterolemia and mixed dyslipidemia to reduce total cholesterol, LDL-C, apo-B, triglycerides levels, and CRP as well as increase HDL levels.

- Heterozygous familial hypercholesterolemia in pediatric patients

- Homozygous familial hypercholesterolemia

- Hypertriglyceridemia (Fredrickson Type IV)

- Primary dysbetalipoproteinemia (Fredrickson Type III)

- Combined hyperlipidemia

Pharmacokinetics:

Absorption: In clinical pharmacology studies in man, peak plasma concentrations of rosuvastatin were reached 3 to 5 hours following oral dosing. Both C_{max} and AUC increased in approximate proportion to CRESTOR® (rosuvastatin calcium) dose. The absolute bioavailability of rosuvastatin is approximately 20%. Administration of CRESTOR with food did not affect the AUC of rosuvastatin. The AUC of rosuvastatin does not differ following evening or morning drug administration.

Distribution: Mean volume of distribution at steady-state of rosuvastatin is approximately 134 liters. Rosuvastatin is 88% bound to plasma proteins, mostly albumin. This binding is reversible and independent of plasma concentrations.

Metabolism: Rosuvastatin is not extensively metabolized; approximately 10% of a radiolabeled dose is recovered as metabolite. The major metabolite is N-desmethylrosuvastatin, which is formed principally by cytochrome P450 2C9, and in vitro studies have demonstrated that N-desmethylrosuvastatin has approximately one-sixth to one-half the HMG-CoA reductase inhibitory activity of the parent compound. Overall, greater than 90% of active plasma HMG-CoA reductase inhibitory activity is accounted for by the parent compound.

Excretion: Following oral administration, rosuvastatin and its metabolites are primarily excreted in the feces (90%). The elimination half-life ($t_{1/2}$) of rosuvastatin is approximately 19 hours. After an intravenous dose, approximately 28% of total body clearance was via the renal route, and 72% by the hepatic route.

Contraindications:

Rosuvastatin has multiple contraindications, conditions that warrant withholding treatment with rosuvastatin, including hypersensitivity to rosuvastatin or any component of the formulation, active liver disease, and elevation of serum transaminases, pregnancy, or breast-feeding. Rosuvastatin must not be taken while pregnant as it can cause serious harm to the unborn baby. In the case of breastfeeding, it is unknown whether rosuvastatin is passed through breastmilk, but due to the potential of disrupting the infant's lipid metabolism, patients should not breast feed while on rosuvastatin.

Adverse effects:

- Constipation, Heartburn, Dizziness, Insomnia, Depression, Joint pain, Memory loss or forgetfulness, Confusion
- The following rare adverse effects are more serious. Like all statins, rosuvastatin can possibly cause myopathy, rhabdomyolysis
- Muscle pain, tenderness, or weakness, Lack of energy, Fever, Nausea, Extreme tiredness, Weakness, Unusual bleeding or bruising, Loss of appetite
- If any signs of an allergic reactions
- Rash, Itching, Difficulty breathing or swallowing, Swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles, or lower legs, Numbness or tingling in fingers or toes.

IV. Methodology:

Animals:

Healthy male wistar albino rats were procured from animal house of Sri Venkateshwara Enterprises, Hyderabad, India and weigh about 120-125gms were used for the study. Every experimental animal was clinically examined pre-operatively for any disease. The animals were kept under observation in laboratory and allowed acclimatize, for 7 days before experimentation. Animals kept in separate spacious clean cages under controlled room temperature $(25\pm1)°c$ & relative humidity $(50\pm15)\%$; in a 12hrs light-dark cycles. They fed with a standard diet and water ad-libitum. Before the experiment the rats were divided into groups

GROUPS:

Group I: Control (Normal control)

Group II : Positive control (5% cholesterol diet for 3months)

Group III: Standard (5% cholesterol diet + ROSVASTATIN (20mg/kg))

Group IV : Test I (Plant Extract-250mg/kg b wt of EEDE+ 5% cholesterol diet)

Group V : Test II (Plant Extract-500mg/kg b wt of EEDE+ 5% cholesterol diet)

PROCEDURE:

Rats were divided into five groups each group contain six animals. Group I serves normal which receives normal saline (5ml/kg b wt) for last 28 days orally; Group II serves as positive control which receives 5% cholesterol diet for 3 months continuously; Group III serves as standard which receives Rosavastatin (20mg/kg b wt; I.P route) for last 28 days and Group IV, Group V serves as test which receives EEDE at dose of 250 and 500 mg/kg b wt, through oral route throughout the study period and also receives 5% cholesterol diet for last 28 days. After the study period animals were sacrificed by collecting blood through cardiac puncture and collected blood was centrifuged and serum was collected and biochemical analysis were

performed lipid profiles for serum levels TC, TG, HDL, LDL, VLDL, Urea, Creatinine, uric acid and antioxidant studies SOD, GSH and Catalase.

Estimation of weight gain:

During the experimental period, the high cholesterol diet consumed and weight gained by rats was recorded on 0th, 14th and 28th day of EEDE treatment. The pre-weighed food pellets (approximately 30g) were placed inside the hopper of the cage. The food consumed by individual rat was quantified by weighing leftover food in the hopper (Kokate et al., 2006).

Estimation of total cholesterol (TC): (CHOD-PAP Method)

The reagents kits intended for the in-vitro quantitative determination of cholesterol in serum/plasma.

Principle: - The cholesterol esters are hydrolysed by enzyme cholesterol esterase to give free cholesterol and fatty acid molicules. This free cholesterol gets oxidised in presence of cholesterol oxidase to liberate cholset 4en-3one and H_2O_2. Liberated H_2O_2 by this reaction combines with phenol and 4 amino antipyrine in presence of peroxidise to form red colourquinonimine complex, the intensity of which is measured at 505 nm. (490-530nm) it is directly proportional to the cholesterol conc. Present in sample.

General system parameters:-

Wave length   : 505 nm (490-530nm)

Incubation   : 5 min

Sample volume   : 10µl

Reagent volume   : 1.0ml

Standard concentration: 200mg/dl

Procedure:-

Bring all the reagents of assay to room temperature.

	Procedure for 1ml			Procedure for 3ml		
	B	**S**	**T**	**B**	**S**	**T**
Enzyme reagent	1 ml	1 ml	1 ml	3 ml	3 ml	3 ml
Standard	-	10µl	-	-	30µl	-
Sample	-	-	10µl	-	-	30µl

Mix well and incubate for 5 min at room temperature. Mix well and measure the absorbance of standard and test against the reagent blank at 505 nm. (490-530nm)

Calculation:-

$$\text{Total cholesterol mg/dl} = \text{Abs TC/Abs STD} \times 200$$

Estimation of triglycerides (TG):(GPO- Method)

Diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.

Principle: The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and FAs. Glycerol then is phosphorylated in an ATP - requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase (GPO) catalyzed reaction. H_2O_2 then reacts with 4 -AAP and 4 - chlorophenol under the catalytic influence of peroxidase to form colouredquinoneimine complex, the intensity of which was measured at 505nm (Venkatesh G1 et al., 2010).

$$\text{Triglyceride} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3 \text{ fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerokinase}} \text{Glycero} -3\text{-phosphate} + ADP$$

$$\text{Glycero} -3\text{-Phosphate} + O_2 \xrightarrow{\text{Glycerophospate oxidase}} DHAP + H_2O_2$$

Reagents used:

Sl. No.	Reagent composition	Conc. in the final test mixed
1.	Pipes butter	50mmol/l
2.	4-Chlorophenol	5mmol/l
3.	Mg^{2+}	5 mmol/l
4.	ATP	1 mmol/l
5.	Lipase	$\geq$5000 U/l
6.	Peroxidase	$\geq$1000 U/l
7.	Glycerol Kinase	$\geq$400 U/l
8.	Glycerol - 3- phosphate oxidase	$\geq$4000 U/l

Standard: The concentration of standard triglyceride used was 200mg/dl

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Reaction parameters:

1	Reaction type	End point
2	Wave Length	505 nm
3	Optical length	1 Cm
4	Temperature	$37\,^{0}$C
5	Measurement	Against reagent blank

Summary of assay details:

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 μl	1000 μl	1000 μl
Standard	-	10 μl	-
Sample	-	-	10 μl

The reaction mixtures were mixed well and incubated for 10 min at $37\,^{0}$C. The absorbance of sample and standard were measured against reagent blank at 505 nm.

Calculations:-

Abs of test

Serum triglycerides (mg/dl) = ⎯⎯⎯⎯⎯⎯ × Conc of standard

Abs of STD

Estimation of high-density lipoprotein cholesterol (HDL-C):

Diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Principle: HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins, intermediate-density lipoproteins directly from serum polyanions like phosphotungstic acid and along with $MgCl_2$ are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is

sedimented by centrifugation and HDL cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation serum of TC (VenuPamidiboina et al., 2010).

Reaction parameters:

1	Reaction type	End point
2	Wavelength	505 nm
3	Optical path	1 cm
4	Temperature	37 ^{0}C
5	Measurement	Against reagent blank

Preparation:-

Take 0.5 ml of serum /plasma in to glass tube. Add 50 µl precipitating reagent. Mix well, leave it at R.T. For 10 min. centrifuge at 3000 r.p.m. for 10 min. take the clean supernatant for HDL cholesterol estimation.

Procedure:-

	B	S	T
Enzyme reagent	1 ml	1 ml	1 ml
Standard	-	0.01 ml	-
Supernatant sample	-	-	0.01 ml

Mix well and incubate for 5 min at 37°c. Measure the absorbance of HDL&std at 510 nm.

Calculations: - HDL cholesterol mg/dl = Abs TH/Abs STD×200

Estimation of creatinine:

Method: Jaffe's method

Principle: Creatinine reacts with alkaline picrate to produce an orange-yellow colour (the jaffe's reaction).The absorbance of orange-yellow coloured complex formation is directly proportional to the creatinine concentration and is measured photo metrically at 500-520 nm (Bowers LD et al., 1980; Bartels H et al., 1972)

Reagents:

1. Picric acid reagent   100ml
2. Alkaline buffer reagent   100ml
3. Standard creatinine   10ml
4. Acid reagent   10ml

Procedure: Set the Auto-analyser instrument with parameters given along with the kit. Prepare the working. Standard, test solution as per the protocol. Mix well and read initial absorbance (A0) 20 seconds after mixing and final absorbance (A1) 80 seconds after mixing at 505 nm.

General properties:

Reaction type	Kinetic with standard
Wave length	520nm (green filter)
Incubation temperature	Room temperature
Incubation time	30 sec
Read time	60 sec
No. of readings	2
Linearity	20 mg%
Sample volume	100μL
Reagent volume	1μL
Cuvette	1cm light path

Laboratory procedure:

	Blank	**Standard**	**Sample**
Working reagent	1ml	1.0 ml	1.0 ml
Standard	_	0.1ml	_
Sample	_	_	0.1ml

Mix well and allow it to stand for 5 mins. Read absorbance for absorbance for standard and sample against distilled water at 520nm against blank.

For test $\Delta AT = A1T-A0T$

For standard $\Delta AS = A1S-A0S$

Calculations: $\dfrac{\Delta AT}{\Delta AS}$

Estimation of urea:

Principle:

Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia released reacts with a mixture of salicylate, hypochlorite and nitropruside to yield a blue-green coloured compound (indophenols). The intensity of colour produced is proportional to the concentration of urea in the sample and is measured photometrically at 570nm or with yellow filter (Chaney AL et al., 1962).

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO2$$
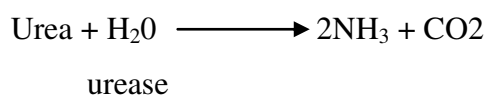
Reagents:

Urease reagent   50ml

Enzyme concentrate 1 vial

Colour reagent   50ml

Urea standard   2ml

General system parameters

Mode of reaction	End point
Wave length	570nm
Temperature	Room temperature
Standard concentration	40mg%
Linearity	300mg/%
Blank	Reagent
Incubation time	10min
Cuvette	1cm light path

Laboratory procedure

	Blank	**Standard**	**Sample**
Working reagent	1000μL	1000μL	1000μL
Standard	_	10μL	_
Sample	_	_	10μL

Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank.

Calculations:

a) Urea concentration mg = $\dfrac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times 40$ (stdconc)

b) Blood Urea Nitrogen in mg% = a $\times$ 0.467

Estimation of uric acid:

Principle:

Uric acid + O_2 + 2 H_2O ———— Allantoine + CO2 + H_2O_2

Uricase

$2H_2O_2$ + ESPAS + 4-AAP ⟶ Purple Quinoeimine + 4 H_2O_2
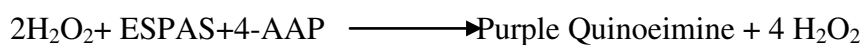
Reagents:

Enzyme reagent   50ml

Standard   2ml

General system parameters

Mode of reaction	End point
Wave length	546nm
Reaction slope	Increasing
Temperature	Room temperature
Standard concentration	5mg%
Linearity	25mg/%
Blank	Reagent
Reagent volume	1ml
Incubation time	10min
Cuvette	1cm light path

Laboratory procedure

	Blank	Standard	Sample
Enzyme Reagent	1000µL	1000µL	1000µL
Standard	_	10µL	_
Sample	_	_	10µL

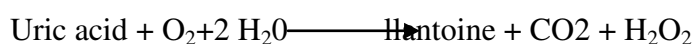Mix and incubate for 5minutes at room temperature. Read the absorbance of the standard and sample against reagent blank (Fossati et al., 1980, Tietz NW et al., 1995, Vassault A et al., 1986).

Calculations:

Uric acid mg/dl   =   <u>Absorbance of sample</u> × 5 (stdconc)

           Absorbance of Standard

Estimation of superoxide dismutase (SOD):

Known amount of tissue was weighed and washed in ice cold saline and homonized in ice cold 0.1M TrisHcl buffer for estimations.

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. 0.05 ml supernatant was added to 2.0 ml of carbonate buffer and 0.5 ml of 0.01mM EDTA solution. The reaction was initiated by addition of 0.5 ml of epinephrine and the autooxidation of adrenaline

(3× 10-4 M) to adrenochrome at pH 10.2 was measured by following change in OD at 480 nm. The change in optical density every minute was measured at 480 nm against reagent blank. The results are expressed as units of SOD activity (mg/wet tissue). One unit of SOD activity induced approximately 50% inhibition of adrenaline.

Estimation of catalase activity (CAT):

Catalase activity was determined spectrophotometrically according to previously published method. Briefly, to 1.95ml of 10mM H_2O_2 in 60mM phosphate buffer (pH=7.0), 0.05ml of the liver homogenate was added and rate of degradation of H_2O_2 was followed at 240nm per min. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula $k=2.303/ \Delta t \times log (A_1/A_2) s^{-1}$ (A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH=7.0 at 25^oC, while H_2O_2 concentration falls from 10.3 to 9.2 mM) [prabakar KR et al., 2006].

Estimation of glutathione (GSH):

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB. Briefly, proteins were precipitated using 10% TCA, centrifuged and 0.5 ml of the supernatant was mixed with 0.2M phosphate buffer (pH 8.0) and 10mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412nm against appropriate blanks. The glutathione content was calculated by using the standard plot under same experimental conditions. Plotting the absorbance against GSH concentration (0.005-0.042 µmoles) gave the standard curve. A linear correlation coefficient ($r^2 = 0.999$) was obtained [Moran A et al., 1979].

4.3 Histopathological studies:

The tissues were washed immediately with saline and then fixed in 10% formalin solution. After fixation, the heart tissues were processed in alcohol- xylene series and then embedded in paraffin. The serial sections were cut and each section

was stained with hematoxylin and eosin. The slides were examined under microscope and photographs were taken.

4.4Statistical analysis:

The result were expressed as mean ± SD and analyzed statistically using one way ANOVA followed by Dunnet test, Data were computed for statistical analysis

5. MATERIALS AND METHODS

5.1. Materials

Table3: List of chemicals

S.NO	Chemical	Manufacture
1	Cholesterol	Mathri Laboratories Pvt. Limited
2	Rosuvastatin	Ranbaxy
3	Formalin	Sd-fine chem.. Limited
4	Cholesterol kit	Span diagnostics
5	Triglyceride kit	Span diagnostics
6	HDL kit	Span diagnostics
7	Urea kit	Span diagnostics
8	Creatinine kit	Span diagnostics
9	Uric acid kit	Span diagnostics
10	Antioxidant studies kit	Span diagnostics

List of equipments

Table5: List of equipment

S.NO	Equipment	Manufacture
1.	Semi auto analyser (CL380 biochemistryanalyser)	Elico
2.	Laboratory Centrifuge (R-8c)	Remi
3.	Micropipette	Thermo scientific
4.	Junior Grindwell	J.U.C. Mumbai

The Present study was designed to assess the anti- hyperlipidemic activity of Delonix Elata.on high cholesterol diet induced hyperlipidemia in rats and the work was carried out using the methods described below:

6. RESULTS

6.1. Extract:

Table-6: Ethanolic extract of aerial parts of *Delonix elata* shows yield, colour, nature.

S.NO	EXTRACT	RESULT
1	YIELD	13.5%
2	COLOUR	Yellowish brown
3	NATURE	Semisolid

6.2. PHYTOCHEMICAL ANALYSIS:

The preliminary phytochemical analysis revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, steroids, saponins, proteins, gums and phenolic compounds in ethanolic extract of aerial parts of *Delonix elata*

Table-7: Preliminary Phytochemical analysis of ethanolic extract aerial parts of *Delonix elata (Consolidated Repord)*

S.NO	COMPOUNDS	EEDE
1	Carbohydrates	+
2	Alkaloids	+
3	Glycosides	+
4	Fixed oils	-
5	Flavonoids	+
6	Tannins	+
7	Steroids	+
8	Phenolic compounds	+
9	Amino acids	-
10	Saponins	+
11	Gums	+
12	Proteins	+

+: presence of compounds

-: absence of compounds

6.3. ACUTE TOXICOLOGICAL STUDY

The acute toxicity of the EEDE was carried out and the results are tabulated below according to procedure was followed by using OECD 423 (Acute Toxic Class Method).

Table -8: Acute toxicity studies of EEDE

S.NO	Groups	Dose/kg (body weight) p.o	Signs of Toxicity	Onset of Toxicity	Duration of Study
1	EEDE	5mg	No signs of Toxicity	Nil	14 days
2	EEDE	50mg	No signs of Toxicity	Nil	14 days
3	EEDE	300mg	No signs of Toxicity	Nil	14 days
4	EEDE	2000mg	No signs of Toxicity	Nil	14 days
5	EEDE	5000mg	No signs of Toxicity	Nil	14 days

The therapeutic dose was calculated as $1/10^{th}$ of the lethal dose for the purpose of antihyperlipidemic activity. The dose of EEDE was further reduced to 2000 mg/kg and observed for 14 days. This particular dose was found to be safe and no toxicity was observed. One-tenth of the upper limit dose was selected as 250 mg/kg b.wt for test group. The LD_{50} value determined by the method 423 as per guidelines of Organization for Economic Co-operation Development (OECD) was found to be 5000mg/kg b.w. by oral route of EEDE which show no toxicity at this
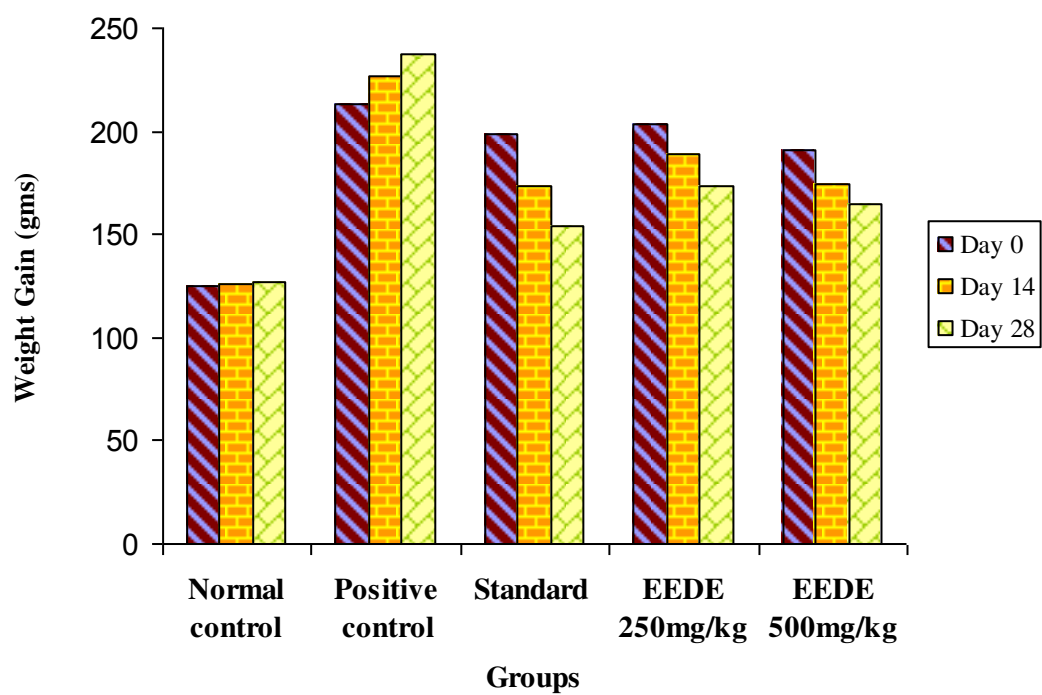
particular dose. Therefore, 1/10 and 1/20 of lethal dose has been selected for the experiment. i.e 250 mg and 500 mg/kg

6.4. HYPERLIPIDEMIA ACTIVITY: HIGH CHOLESTEROL DIET INDUCED HYPERLIPIDEMIC ACTIVITY

TABLE-9: Effect of EEDE on weight gain in hyperlipidemia-induced rats for 12 weeks.

Parameter	Test group	Day 0	14th day	28th day
Weight gain	Normal control	124.7±3.10	126± 3.36	126.5± 3.12
	Positive control	213.5±7.00	226.8±8.21	237.8±7.22
	Standard	198.3±4.79***	173.3± 5.03***	154±4.55***
	EEDE250mg/kg	203.5±5.36*	189±7.05***	173.7±5.01***
	EEDE500mg/kg	191.3±3.78***	174.2±4.68***	165±4.87***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, ** P<0.01, ***P<0.001 (one way ANOVA followed by Dunnett test)

Graph I

**Effect of EEDE on weight in in cholesterol induced rats.**

Values are expressed in Mean± SD for six animals.

TABLE 10: Effect of EEDE on serum lipid profile

Groups	Serum cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal control	66.23± 3.98	58.21± 2.40	37.97± 4.8	17.91± 2.60	12.43± 2.16
Positive control	170.58± 10.57	145.8± 8.18	21.67±2.80	118.3± 7.16	28.67± 3.733
Standard	110.5± 3.73***	95.83± 3.31***	33.83± 3.18***	48.50± 3.55***	18.73± 2.51***
EEDE 250mg/kg	136.9± 4.16***	120.00± 4.74***	27.47± 2.60^{ns}	87.00± 3.23***	21.70± 2.739**
EEDE-500mg/kg	122.5± 4.88***	107.8± 3.76***	31.5± 2.73*	68.33± 2.58***	21.83± 3.18***

Values are expressed as mean ± SD (n=6).Values were significant when compared

with cholesterol group. * $P<0.05$, ** $P<0.01$, ***$P<0.001$ (one way ANOVA

followed by Dunnet test)

Graph II Effect of EEDE on lipid profiles TC, TG, LDL, VLDL, HDL levels in cholesterol induced rats
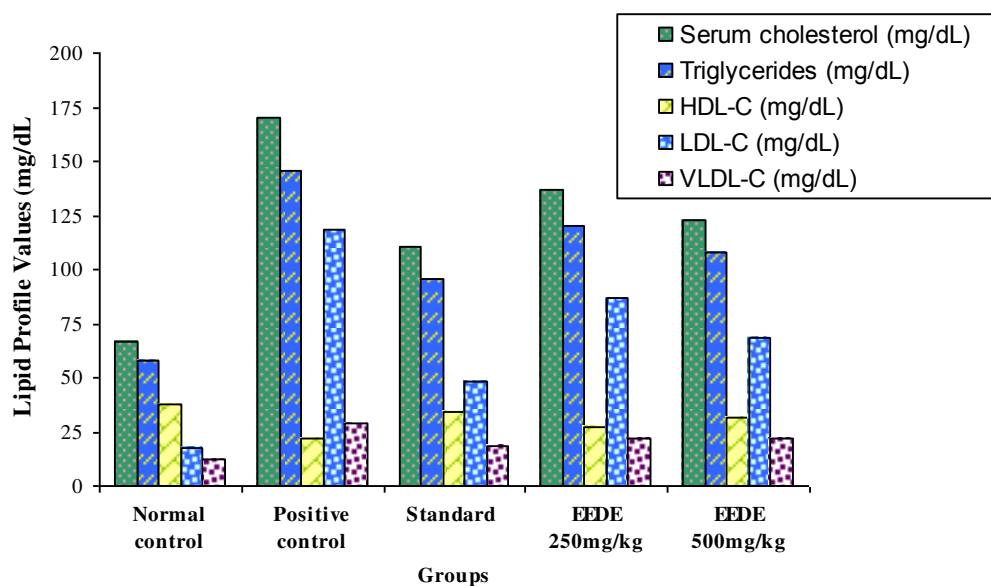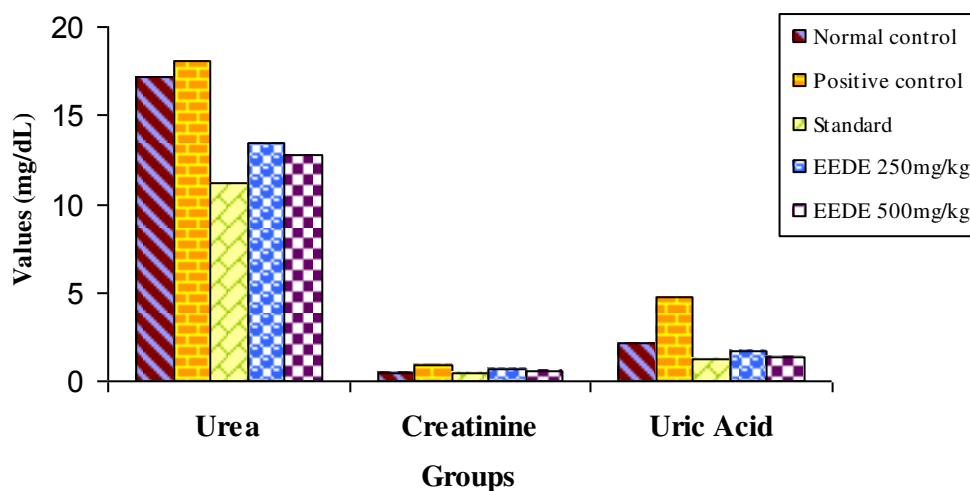

Values are expressed in Mean for six animals.

TABLE-11: Effect of EEDE on serum kidney biomarkers in normal and cholesterol rats.

GROUPS	UREA (mg/dL)	CREATININE(mg/dL)	URIC ACID (mg/dL)
Normal control	17.2±2.30	0.43±0.101	2.1±0.50
Positive control	18.1±3.2	0.85±0.12	4.80±1.4
Standard	11.2±1.70***	0.44±0.108***	1.29±0.10***
EEDE-250mg/kg	13.5±0.80*	0.63±0.09*	1.69±0.51***
EEDE-500mg/kg	12.8±0.60*	0.61±0.07*	1.38±0.23***

Values are expressed as mean ± SD (n=6).Values were significant when compared with positive control group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet test).

Graph III: Effect of EEDE on serum kidney biomarkers urea, creatinine, uric acid levels in cholesterol induced rats.
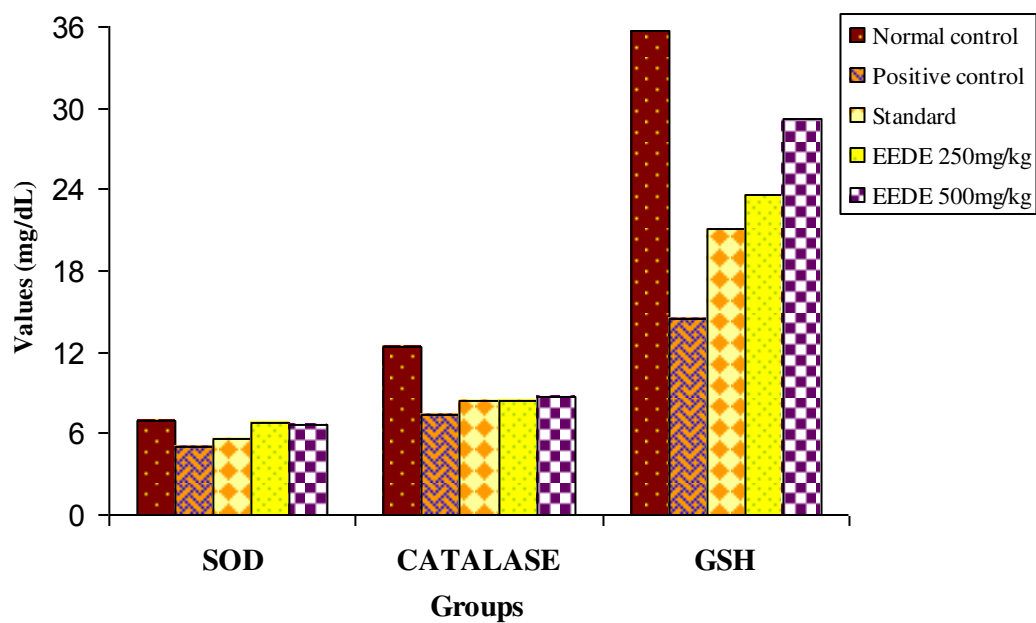

Values are expressed in Mean± SD for six animals.

TABLE-12: Effect of EEDE on antioxidant enzymes in control and experimental rats.

| GROUPS	SOD	CATALASE	GSH
Normal control	6.89± 0.60	12.38± 0.81	35.76±1.16
Positive control	5.00± 0.61	7.31± 0.69	14.39±0.56
Standard	5.64± 0.72*	8.36± 0.64[ns]	21.12±0.98***
EEDE 250mg/kg	6.72± 0.87***	8.41± 0.75**	23.54±1.16***
EEDE 500mg/kg	6.61± 0.63***	8.73± 0.60***	29.27±1.87***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet test).

Graph IV: Effect of EEDE on antioxidants of SOD, CAT and GSH levels in cholesterol induced rats

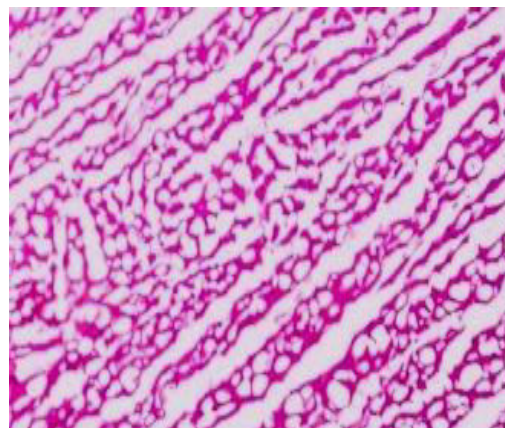Values are expressed in Mean± SD for six animals.
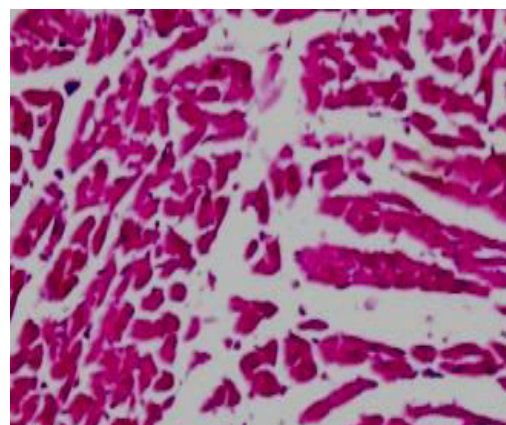
5.5. Histopathological studies
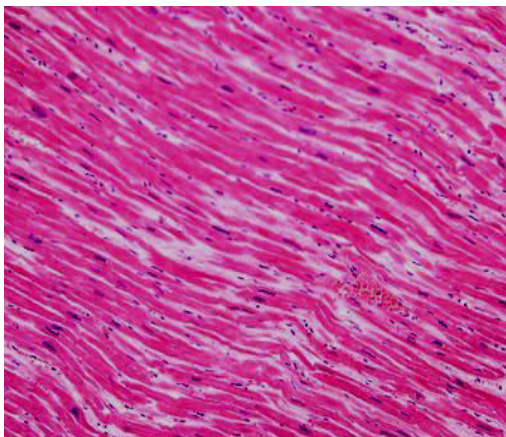
Normal control of Heart 10X

Positive control Heart 10X

Standard group of Heart 10X

EEDE 250mg/kg of heart 10X

EEDE 500mg/kg of heart 10X

7. DISCUSSION

Hyperlipidemia means presence of a high amount of cholesterol in the body has been demonstrated to elevate total cholesterol and may increase the risk of atherosclerosis and lead to cardiovascular disease. Herbs play a key role in the management of various CVD. Numerous medicinal plants and their formulations are used for hyperlipidemia in ethnomedical practices and in traditional system of medicine in India. However, we do not satisfactory remedy for hyperlipidemia; most of the herbal drugs speed up the reduction of cholesterol by healthy dietary intake. So, the search for anti-hyperlipidemic activity of high cholesterol induced rats.

The extraction by soxhlet method for 100gms of powder yielded 12.8%. The preliminary phytochemical screening revealed the presence of Carbohydrates, Alkaloids, Glycosides, Flavonoids, Tannins, Phenolic compounds, Amino acid, Saponins, Steroids, Gums and Proteins [Tijare R D et al., 2012]. The extract was found safe up to a dose of 5000mg/kg body weight. The dried extract was suspended in 1% CMC at dose levels 250mg/kg and 500mg/kg body weight for oral administration.

Cholesterol powder was purchased and used with normal diet shows changes in serum cholesterol levels differ significantly between rats and humans [Suckling KE et al., 1993]. To clarify these confusions, a 12 week's feeding study with rats was conducted to evaluate the influence of high fat diet on serum lipid profiles, kidney parameters and antioxidant studies.

The use of rats as experimental animals for hyperlipidemic activity is mainly due to the synthesis and structural similarities in human and rats.

The present study carried out inducing 5% cholesterol for the experimental induction in rats. Cholesterol will deposits in the endothelial cells and transport with lipoproteins in blood stream which leads to increase in the TC, TG, LDL, VLDL levels and decreases the HDL levels in the body. It is synthesised in the liver and converted into bile acids and excreted through faeces and due to hypercholestremia urine failure will occur due to oxidative stress.

Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Rosvastatin is a competitive inhibitor of HMG-CoA reductase and catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) to mevalonate, which is the rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases hepatic cholesterol, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes and decrease the amount of LDL-cholesterol in the blood. Like other statins, rosvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol [Villa J et al., 2010 and McCormack T et al., 2010].

The assessment of cholesterol function can be made by estimating the body weight and activities of various lipid profiles such as TC, TG, HDL, LDL and VLDL; kidney parameters such as urea, creatinine and uric acid.

Body weight can be increased due to the high cholesterol diet induced in hyperlipidemia rats. As the dietary fat, FFAs can be synthesised into many tissues by transport of increased lipoproteins in the blood stream absorbed from the intestine and metabolised in liver [Van Heek et al., 2000]. The EEDE treatment and Rosvastatin drug significantly lower the body weight by the reduction in the LDL, VLDL levels and increase HDL levels.

TC and TG are present in dietary fat, FFAs combine with glycerol to form TG and cholesterol is esterified by ACAT to form cholesterol esters, storing cholesterol in cells [Lombardo et al., 1980]. TC and TG are generated by the liver and circulate as chylomicrons interact at capillaries of adipose tissues and muscle cells. LPL hydrolyzes the TG, and FFAs are released. The elevated TC and TG levels in hyperlipidemia induced high cholesterol diet are due to the increase of cholesterol in capillary cells. The EEDE treatment and Rosvastatin drug significantly lowered the abnormal levels of TC and TG might be due to lowering of FFA (Free fatty acids) synthesis in the plasma.

LDL and VLDL are package of TG and TC esters in liver and released into blood circulation. VLDL is then hydrolyzed by LPL in tissues to release fatty acids

taken up by muscle cells for energy and glycerol becomes a VLDL remnant taken up by the liver by LDLR. They hydrolyzed in the liver by HL to form LDL. LDLR activity and uptake regulates plasma LDL concentration through decreasing the synthesis of HMG-CoA reductase controls the rate of de novo cholesterol synthesis by the cell [Sabrin R. M. Ibrahim et al., 2013]. LDL and VLDL levels are increased due to the suppression of synthesis of new LDLR in the cells, activates the enzyme ACAT, free cholesterol into cholesterol ester by cholesterol diet. The EEAG and Rosuvastatin treatment significantly decrease the LDL and VLDL levels in the liver.

HDL is a key lipoprotein involved in reverse cholesterol transport and transfer of cholesterol esters between lipoproteins and secreted by the liver and intestine proceeds through a series of conversions known as the HDL cycle. It attracts cholesterol from cell membranes and free cholesterol to the core of the HDL particle, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidizes LDL [Parthasarathy S et al., 1990]. The EEDE and Rosvastatin treatment significantly increase the HDL levels due to the synthesis of the liver by converting bile into bile acids and excreated through faeces by decrease TC and TG levels. Flavonoids can increase HDL-C and also decreases oxidation of LDL- cholesterol [Akila M et al., 2008].

Urea, uric acid and creatinine levels were increase due to the high cholesterol diet [Sudhahar et al., 2008]. There is a association between hypercholesterolemia and kidney damage in which the oxidative stress and inflammatory responses are involved in renal injury was up regulated by hypercholesterolemic condition. Oxidative stress might play a patophysiological role due to increase of MDA levels present in higher lipid group due to impaired pro-oxidant and antioxidant mechanism [Kumari S.S et al., 1987]. Altered physical properties of cellular membrane may facilitate the escape of free radicals from mitochondrial electron transport chain leads to lipid peroxidation and cell oxidative injury [Engelmann B et al., 1992 and Ludwig P.W et al., 1982].Further hypercholesterolemia associated with oxidative modification of LDL, Protein glycation, glucose auto oxidation leads to elevated oxidative stress. The treatment with EEDE and rosavastatin significantly decrease the levels of urea, uric acid and creatinine when compare to hyperlipidemia group.

Antioxidant defence enzymes SOD, CAT and GSH, protect the aerobic cells against oxygen stress and lipid peroxidation. SOD and GSH are the first line of cellular defence against oxidative injury by excess free radical production which is involved in the disposal of superoxide radical to hydrogen peroxide and catalase converts H_2O_2 to H_2O. Low SOD and GSH activity attribute inactivation of enzyme by ROS bringing damage to proteins. GSH is a stable enzyme inactivated by severe oxidative stress [Condell R.A et al., 1983] due to hyperlipidemia. The declined levels of lipid peroxidation in EEDE and rosavastatin treated rats contribute to the potential inhibitors of lipid peroxidation due to the presence of polyphenols like flavonoids, tannins and phenolics as they are reported to exhibit antioxidant properties.

The histological studies reveals that the heart sections treated/induced with cholesterol showed a marked fat cells in the heart with can be increase the lipid peroxidation due to oxidative stress causes increase the TC, TG, LDL, VLDL levels in positive group when compare to normal group result decrease levels of lipoproteins and show the normal architecture of heart cells.

Animals treated with rosovastatin showed a significant restoration of lipoproteins in the heart by maintaining ROS production whereas group IV and V animals treated with EEDE preserved the architecture of heart cells by reducing the free fatty acids exhibiting antihyperlipidemic activity.

Naturally occurring flavonoids and saponins can reduce the excess production of LDL and VLDL levels and increase the HDL levels in the tissues.

8. SUMMARY & CONCLUSION

The aim of the study is to evaluate the antihyperlipidemic activity of the ethanolic extract of aerial part of *Delonix elata* in cholesterol diet induced hyperlipidemic rats.

Wistar albino rats were randomly divided into five groups of six each. Group-I served as normal control. Groups II to V were given 5% cholesterol diet for 3 months to induce hyperlipidemia, and for last 28 days were administered either: 0.5ml water/saline for Group- I; cholesterol diet (5%) for Group-II; Standard drug Rosuvastatin (20mg/kg body weight) for Group-III; EEDE at 250 mg/kg bodyweight for Group-IV and 500mg/kg body weight for Group-V. The effects of EEDE on Body Weight, serum lipid profile, kidney parameters and anti oxidant enzymes (superoxide dismutase, GSH and catalase) were assessed and compared.

Cholesterol diet induced hyperlipidemic rats showed an significant ($P<0.001$) increase in the plasma concentration of Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein cholesterol (LDL-c), Very Low-Density Lipoprotein cholesterol (VLDL-c), Body weight and kidney parameters; it decrease in High Density Lipoproteins Cholesterol (HDL-c) and antioxidant enzymes when compared to normal control rats. Co-administration of EEDE and standard drug Rosuvastatin with cholesterol diet caused a significant decrease ($p<0.001$) in the concentration of serum TC, VLDL, TG, body weight and kidney parameters; increase in the HDL-C and antioxidant enzymes when compared with cholesterol fed control rats. The result suggests lipid lowering effects of *Delonix elata* which serves as a new potential natural product for preventing hyperlipidemia.

In this conclusion, the findings of the study suggest that *Delonix elata* is a potent for antihypercholesterolemic, antihypertriglycerolemic drug lowering LDL, VLDL and increasing HDL levels in all the High cholesterol diet treated groups. The mechanism has point towards inhibiting cholesterol and triglyceride synthesis. Aerial parts of *Delonix elata* proved to be effective at higher doses (500mg/kg) and Rosuvastatin standard drug shows better results in decrease TC, TG, LDL and

VLDL levels and increase the HDL levels compare to hyperlipidemia rats. The urea, creatinine and uric acid levels are decreased with EEDE compare to the hyperlipidemia rats. In hyperlipidemic rats, the increases of antioxidant enzymes-SOD, CAT and GSH due to the free radical mechanism which could be reduces with help of EEDE groups. Therefore, it can be recommended for further investigation to reveal the exact mechanism for hyperlipidemia activity.

9. REFERENCES

1   Abd El G, Hegazi M. In vitro studies on *Delonix elata* L.-an endangered medicinal plant. World Applied Science J, 2011; 14(5): 679-686.

2   Ayinla MT, Dada SO, Shittu ST, Olayaki LA, Akiode AO and Ojulari SL. Anti-hyperlipidemic effect of aqueous leaf extract of *Ocimum gratissimum* in alloxan induced diabetic rats. *International Journal of Medicine and Medical Sciences* 2011; 3(12): 360-363.

3   Anreddy RNR, Porika M, Yellu NR, Devarakonda RK. Hypoglycemic and Hypolipidemic Activities of *Trianthema portulacastrum* Linn. Plant in Normal and Alloxan Induced Diabetic Rats. *International Journal of Pharmacology* 2010; 6(2): 129-133.

4   Ahmed, S.M., Clasen, M.D., and Donnelly, M.D: Management of dyslipidemia in adults, *American Family Physician,* 57(9), 1998, 1-16.

5   American Heart Association: Scientific statement on, *Cholesterol and Hyperlipidemia,* 2005, pp 1-2.

6   Aminoff, M.J, Tjerney, L.M, Nervous system, *In Current Medical Diagnosis and Treatment*, The McGraw-Hill company, 43rd edition, pp 956-963 (2004).

7   Abderrahmane Aissaoui, Soumia Zizi, Zafar H. Israili, Badiaa Lyoussi, Hypoglycemic and hypolipidemic effects of *coriandrum sativum* L. in Meriones shawi rats, *Journal of Ethanopharmacology 137,* 2011, 652-661.

8   A Trovato, M.T. Monforte, R. Barbera, A. Rossitto, E.M. Galati, A.M. Forestieri, Effects of fruit juices of Citrus sinensis L. and Citrus limon L. on experimental hypercholesterolemia in the rat, *Phytomedicine Vol. 2 (3)* 1996, pp. 221-227.

9   Akila M, Devaraj H. Synergistic effect of tincture of crataegus and *Mangiferaindica* L. Extract on hyperlipidemic and antioxidant status in atherogenic rats. *VascPharmacol* 2008; 49: 173-177.

10 Bottcher H, Miracle drugs, Zagreb: *Zora;* 1965, pp: 23–139.

11 Bachorik, P.S., Denke, M.A., Stein, E.A., and Rifkind, B.M: Lipid and Dyslipoproteinemia, *In Clinical Diagnosis and Management by Laboratory Methods*, 29th edition, Vol-1, 2001, pp 224-248.

12   Baron, R.B: Lipid Abnormalities, *In Current Medical Diagnosis and Treatment*, The McGraw-Hill Company, 44th edition, 2005, 1202-1213.

13   B.V. Ghule, M.H. Ghante, A.N. Saoji, P.G. Yeole, Antihyperlipidemic effect of the methanolic extract from Lagenaria siceraria Stand. fruit in hyperlipidemic rats, *Journal of Ethnopharmacology 124,* 2009, 333-337.

14   B Priyanka, Jyothi M Joy, G Avinash Kumar, S Mohana Lakshmi, Comparitive antioxidant activity of *Asparagus racemosus* with its alternative source *Asparagus gonoclados*, *International Journal of Pharmacotherapy 2(2)*, 2012, 51-56.

15   Bowers LD et al., 1980.Clin.Chem.26:665.

16   Bartewls H & et al., 1972.Clin.Chem.Acta.37, 193.

17   Chaney AL and Marbach EP 1962.Clin.Chem.8. 130.

18   Condell R.A. Evidence for suitability of glutathione peroxidase as a protective enzyme: studies of oxidative damage, restoration and proteolysis. Arch. *Biochem. Biophys.* 1983; 223:407–416.

19   Drug Evaluation Annual: Published by the *"American Medical Association",* 1995, pp 2455-2500.

20   El-Desoky GE, Aboul-Soud MAM, Al-Numair KS. Antidiabetic and hypolipidemic effects of Ceylon cinnamon (*Cinnamomum verum*) in alloxan-diabetic rats. *Journal of Medicinal Plants Research* 2012; 6(9): 1685-1691.

21   Engelmann B. Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. I. Increased phosphatidylcholine and reduced sphingomyelin in patients with elevated levels of triacylglycerol-rich lipoproteins. *Biochim. Biophys. Acta.* 1992; 1165:32–37.

22   Fossati. Principle L., Berti G. *'Clin.Chem.''*26.227(1980)

23   Gayathri V, Ananthi S, Vasanthi HR. Antihyperlipidemic Potential of Polyphenol and Glycoside Rich *Nerium oleander* Flower against Triton WR-1339-Induced Hyperlipidemia in Experimental Sprague Dawley Rats. *Journal of Chemistry* 2013; 1-8.

24   Ginsberg, H.N and Goldberg, IJ: Disorders of Intermediary Metabolism (Disorders of lipoprotein metabolism), In *principles of Internal Medicine,*15th edition, 2001.

25   Gehard Vogel H, Drug discovery and evaluation-Pharmacological assays, 2nd edition, Berlin: Spinger-Verlag; 2001, P.1095-1125.

26   Husain GM, Chatterjee SS, Singh PN, Kumar V. Hypolipidemic and Antiobesity-Like Activity of Standardised Extract of *Hypericum perforatum* L. in Rats. *ISRN Pharmacology* 2011; 1-7.

27   Hossain MS, Ahmed M, Islam A. Hypolipidemic and hepatoprotective effects of different fractions of methanolic extract of *Momordica charantia* (Linn.) in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences and Research* 2011; 2(3): 601-607.

28   Hicham Harnafi, Hana Serghini Caid, Nour el Houda Bouanani, Mohammed Aziz, Souliman Amrani, Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in Triton WR-1339-induced hyperlipidemic mice, *Food Chemistry 108,* 2008, 205-212.

29   Hermann M, Bogsrud MP, Molden E, Asberg A, Mohebi BU, Ose L, Retterstøl K, "Exposure of atorvastatin is unchanged but lactone and acid metabolites are increased several-fold in patients with atorvastatin-induced myopathy". *Clin. Pharmacol. Ther.* **79** (6): 2006, 532–9.

30   Jeong-Sun Lee, Song-Hae Bok, Seon-Min Jeon, Hye-Jin Kim, Kyung-Min Do, Yong-Bok Park, Myung-sook Choi, Antihyperlipidemic effects of buckwheat leaf and flower in rats fed a high-fat diet, *Food chemistry 119,* 2010, 235-240.

31   Kamalraj R. Antihyperlipidemic studies on leaf extract of *Erythrina indica* lam. *International Journal of Research in Pharmacy and Chemistry* 2011; 1(3): 488-490.

32   Kelly K, History of medicine, New York: Facts on file; 2009, pp: 29–50.

33   Kiritikar K. Basu B. Indian Medicinal Plants. 1999;2(2):852

34   Kokate et al ., 2006, Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, 16th ed, Pune: Nirali Prakashan 2006.p. 149-156.

35   Kumud Kumari, K.T. Augusti, Lipid lowering effect of S-methyl cysteine sulfoxide from *Allium cepa* Linn in high cholesterol diet fed rats, *Journal of Ethnopharmacology 109,* 2007, 367-371.

36 Kumari S.S. Changes in concentration of lipid peroxides and activities of superoxide dismutase and catalase in isoproterenol induced myocardial infarction in rats. *Indian J. Exp. Biol.* 1987;25:419–423.

37 Ludwig P.W. Increased leucocyte oxidative metabolism in hyperlipoproteinaemia. Lancet. 1982;8294:348–350.

38 Lakshmi V, Sonkar R, Khanna AK. Antihyperlipidemic and antioxidant activities of *Bruguiera cylindrinca* (L). Chron Young Sci 2012; 3(3): 236-43.

39 Laszlo Czako, Annamaria Szabolcs, Agota Vajda, Sandor Csati, Viktoria Venglovecz, Zoltan Rakonczay, Peter Hegyi, Hyperlipidemia induced by a cholesterol-rich diet aggravates necrotizing pancreatitis in rats, *European Journal of Pharmacology 572*, 2007, 74-81.

40 Li-Jun Feng, Chen-Huan Yu, Ke-Jing Ying, Jian Hua, Xiao-Yan Dai, Hypolipidemic and antioxidant effects of total flavonoids of perilla Frutescens leaves in hyperlipidemia rats induced by high-fat diet, *Food Research International 44,* 2011, 404-409.

41 McCormack T, Harvey P, Gaunt R, Allgar V, Chipperfield R, Robinson P (July 2010). "Incremental cholesterol reduction with ezetimibe/simvastatin, atorvastatin and rosuvastatin in UK General Practice (IN-PRACTICE): randomised controlled trial of achievement of Joint British Societies (JBS-2) cholesterol targets*". Int. J. Clin. Pract.* 64 (8): 1052–61.

42 Maniyar Y, Bhixavatimath P. Antihyperglycemic and hypolipidemic activities of aqueous extract of *Carica papaya* Linn. leaves in alloxan-induced diabetic rats. *J Ayurveda Integr Med* 2012; 3(2): 70-74.

43 Mallick C, Maiti R, Ghosh D. Comparative Study on Antihyperglycemic and Antihyperlipidemic Effects of Separate and Composite Extract of Seed of *Eugenia jambolana* and Root of *Musa paradisiaca* in Streptozotocin-Induced Diabetic Male Albino Rat. *Iranian Journal of Pharmacology & Therapeutics* 2006; 5(1): 27-33.

44 Makwana MV, Pandya NM, Doshi BD, Darji DN, Desai SA, Bhaskar VH. Evaluation of antioxidant and antihyperlipidemic potential of *Sida cordifolia linn.* in experimental animals. *International Journal of Pharmacy* 2012; 2(3): 520-525.

45   **M Angeles Zulet, Ana Barber, Henri Garcin, Paul Higueret and José Alfredo Martinez.** Alterations in Carbohydrate and Lipid Metabolism Induced by a Diet Rich in Coconut Oil and Cholesterol in a Rat Model, *Journal of the American College of Nutrition*, 1999, 18(1): 36-42.

46   Mehdi Harati, Mohsen Ani and Manoochehr Messripour. Effect of Vandyl Sulfate on Fructose –Induced Insulin Resistance Rat. *Iranian biomedical journal*, 7(4) 2003, p:179-182.

47   Marshall, W.J: Lipids and Lipoproteins- *In Illustrated Text Book of Clinical Chemistry*, 2nd edition, Gower Medical Publishing, London, 1992, pp 222-237.

48   Mahley, R.W and Bersot, T.P.: Drug Therapy for Hypercholesterolemia and Dyslipidemia, In *"Goodman & Gilman's – The Pharmacological basis of Therapeutics* (Eds: Hardman, J.G and Limbird, L.E), 10th edition, McGraw-Hill companies, Inc, N.Y, 2001, pp 971-1002.

49   Massie, B.M and Amidon, T.M: Heart – In, *"Current Medical Diagnosis and Treatment"* (Eds. Tierney, L.M. et al.,) The McGraw-Hill Company, 43rd edition, 2004, pp 306-400.

50   Madhava Chetty K. et al., Flowering plants of Chittor District, Student offset printers, Tirupati, Andrapradesh, India, 2008, pp 505.

51   Nainwal P, Dhamija K, Tripathi S. Study of antihyperlipidemic effect on the juice of the fresh fruits of *Lagenaria siceraria*. *International Journal of Pharmacy and Pharmaceutical Sciences* 2011; 3(1): 88-90.

52   Punitha D, Thandavamoorthy A, Arumugasamy K, Suresh SN, Danya U, Udhayasankar RM. Anti-hyperlipidemic effect of ethanolic leaf extract of *Gmelina arborea* in streptozotocin induced male wistar albino rats. *International Journal of Life Sciences and Pharma Research* 2012; 2(3): 46-51.

53   Pragda SS, Kuppast IJ, Mankani KL, Ramesh L. Evaluation of antihyperlipidemic activity of leaves of *Portulaca oleracea linn* against dexamethasone induced hyperlipidemia in rats. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4(4): 279-283.

54   P.R Verma, S.A. Deshpande, Y.N. Kamtham, L.B. Vaidya, Hypolipidemic and antihyperlipidemic effects from an aqueous extract of Pachyptera hymenaea (DC.) leaves in rats, *Food Chemistry 132*, 2012, 1251-1257.

55   Pannerselvam vijayaraj, Kannan Muthukumar, Jayaraja Sabarirajan, Vasanthi Nachiappan*, Antihyperlipidemic activity of cassia auriculata flowers in triton WR 1339 induced hyperlipidemic rats, *Experimental and Toxicologic Pathology 65*, 2013, 135-141.

56   Qureshi SA, Nawaz A, Udani SK, Azmi B. Hypoglycaemic and Hypolipidemic Activities of *Rauwolfia serpentina* in Alloxan-Induced Diabetic Rats. *International Journal of Pharmacology* 2009; 5(5): 323-326.

57   Ramesh Babu K, M Dilip Rajasekhar, Vinay Kumar K, Shaik Sameena Fatima, E Guravaiah Tiruvenkata Kumar, Sirasanagandla Swapna, Bellamkonda Ramesh, Chippada Appa Rao*, Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of Syzygium Alternifolium seeds in streptozotocin induced diabetic rats, *Food and Chemical Toxicology 48,* 2010, 1078-1084.

58   Rajesh R, Chitra K, Padmaa M Paarakh, Anti hyperglycemic and antihyperlipidemic activity of aerial parts of *Aerva lanata* Linn Juss in streptozotocin induced diabetic rats, *Asian Pacific Journal of Tropical Biomedicine*, 2012, S924-S929.

59   Ramdas Pandhare, Sangameswaran Balakrishnan, Popat Mohite, Shnataram Khanage, Antidibetic and antihyperlipidaemic potential of *Amaranthus viridis* (L.) Merr. In streptozotocin induced diabetic rats, *Asian Pacific Journal of Tropical Disease,* 2012, S180-S185.

60   Rajalakshmy I, RamyaPydi S, Kavimani, Cardioprotective medicinal plants-A review, *international journal of pharmaceutical invention*, 2011,vol3, pp:24-41.

61   Rajani GP, Ashok P. *In vitro* antioxidant and antihyperlipidemic activities of *Bauhinia variegata* Linn. *Indian Journal of Pharmacology* 2009; 41(5): 227-232.

62   Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal plants, vol. 2, 1970-1979, 2nd Reprint, NISCAIR, Newdelhi and CDRI, Lucknow, 1999, p. 80.

63   Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal plants, vol. 3, 1980-1984, Reprint, NISCAIR, Newdelhi and CDRI, Lucknow, 2001, p. 76.

64   Reszka, A.A., & Rodan, G.A. Bisphosphonate mechanism of action. *Current Rheumatology Reports,* 2003 *5*(1), 65-74.

65   Schmidt, E.B., & Larsen, M.L. HMG-CoA-reductase-inhibitors. In S. Offermanns, & W. Rosenthal (Eds.). *Encyclopedic reference of molecular pharmacology* 2004 (pp. 460-463). Berlin, Germany: Springer.

66   Shivali, Mahadevan N, Kamboj P. Antihyperlipidemic effect of hydroalcoholic extract of Kenaf (*Hibiscus cannabinus* L.) leaves in high fat diet fed rats. *Annals of Biological Research* 2010; 1(3): 174-181.

67   Stojanoski N, Development of health culture in Veles and its region from the past to the end of the 20th century Veles, *Society of science and art*, 1999, 13–34.

68   Srivastava B, Sinha AK, Gaur S. Barshiliya Y. Study of hypoglycaemic and hypolipidemic activity of *Eugenia Jambolana* pulp and seed extract in Streptozotocin induced diabetic albino rats. *Asian Journal of Pharmacy and Life Science* 2012; 2(1): 10-19.

69   Shah KA, Patel MB, Shah SS, Chauhan KN, Parmar PK, Patel NM. Antihyperlipidemic activity of *Mangifera indica* l. leaf extract on rats fed with high cholesterol diet. Der Pharmacia Sinica 2010; 1(2): 156-161.

70   Selley, R.R., Stephens, T.D., Tate, P: "*Anatomy and Physiology*", 5th edition, McGraw Hill Companies Inc, 2000 pp 646-652.

71   Sinnathambi Arulmozhi, Papiya Mitra Mazumder, Sathiyanarayanan Lohidasan, Prasad Thakurdesai, Antidiabetic and antihyperlipidemic activity of leaves of Alstonia scholaris Linn. R.Br., *European Journal of Integrative Medicine 2,* 2010, 23-32.

72   Sini KR, Sinha BN, Karpagavalli M Determining the antioxidant activity of certain medicinal plants of attapady, (Palakkad), India using DPPH Assay, Current Botany 2010;1(1): 13-7.

73   Sivanarayan V, Suriyavathana. Preliminary studies phytochemical and anti microbial activity of Delonix elata and Prosopis cineraria. International journal of current Research. 2010; 8:66-9

74   S. Rashmi Kaup, Nayanatara Arunkumar, Leigelin Kavitha Bernhardt, Rakesh Gorantla Vasavi, Sandeep Sanjeev Shetty, Sheila Ramesh Pai, B. Arunkumar, Antihyperlipedemic activity of *Cynodon dactylon* extract in high-cholesterol diet fed Wistar rats, *Genomic Medicine, Biomarkers, and Health Sciences 3,* 2011, 98-102.

75   Sridevi M, Kalaiarasi P, Pugalendi KV, Antihyperlipidemic activity of alcoholic leaf extract of *solanum surattense* in streptozocin-diabetic rats, *Asian Pacific Journal of Tropical Biomedicine*, 2011, S276-S280.

76   Su Jeong Ban, Catherine W. Rico, In Chul Um, Mi Young Kang, Antihyperlipidemic effects of hydroxyethyl methylcellulose with varying viscosity in mice fed with high fat diet, *Food Research International 48,* 2012, 1-6.

77   Sharma PC, Yelne MB and Dennis TJ, Database on Medicinal Plants used in ayurveda, Vol. 1, *CCRAS*, New Delhi, 2000, p. 418.

78   Tucakov J, Healing with plants − phytotherapy, Beograd: Culture; 1971, pp: 180–90.

79   Tamas Csont, Gabor Balogh, Csaba Csonka, Imre Boros, Ibolya Horvath, Laszlo Vigh, and Peter Ferdinandy, Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts, *Biochemical and Biophysical Research communications 290,* 2002, 1535-1538.

80   Tijare R D, Beknal A B, Mahurkar N, Chandy V, Pharmacognostical and phytochemical studies of Root tubers of *Asparagus gonoclados* Baker., *International Journal of Pharmacognosy and Phytochemical Research 4(3),* 2012, 142-145.

81   Tijare RD, Beknal AK, Mahurkar N, Chandy V, Pharmacological screening of root tubers of *Asparagus gonoclados* Baker for in-vivo Anticancer activity, *Indian pharmaceutical Congress Association,* 2012, 144.

82   Tietz NW. Clinical guide to laboratory tests, 3 th Ed, 1995,624.

83   Vassault A et al., Protocole de validation de techniques. (Document B,stade 3) *Ann.Biol.Clin.* (1986), 44,686.

84   Venkatesh G1, Baburao K2, Rajesh babu M2 , Dhanalakshmi S3,Indira priya darshini, Anti Urolithiatic and anti hyperlipidemic activity of Coleus aromaticus An explanation of the underlying mechanisms, *International Journal of Phytomedicine 2*,3 2010, 284- 291.

85   Venu Pamidiboina[1], Rema Razdan[2], M.G.Hariprasad[2], Evaluation of the antihyperlipidemic, cardioprotective activity of a polyherbal formulation, *International Journal of Pharmacy and Pharmaceutical Sciences vol 2,* 2010, pp. 86 -91.

86   Vikarant Arya and Vivek Gupata, Chemistry and pharmacology of plant cardioprotectives, A Review,*international journal of pharmaceutical sciences and research*, 2011, vol 2(5), pp:1156-1167.

87   Vadivelan R, Dipanjan M, Umasankar P, Dhanabal SP, Satishkumar MN, Antony S, et al. Hypoglycemic, antioxidant and hypolipidemic activity of *Asparagus racemosus* on streptozotocin-induced diabetic in rats. *Advances in Applied Science Research* 2011; 2(3): 179-185.

88   VR Santhosh Kumar, Md Naseeruddin Inamdar, Nayeemunnisa, GL Viswanatha, Protective effect of lemongrass oil against dexamethasone induced hyperlipidemia in rats: possible role of decreased lecithin cholesterol acetyl transferase activity, *Asian Pacific Journal of Topical Medicine,* 2011, 658-660.

89   Venkatakrishnan Kamesh, Thangarajan Sumathi*, Antihypercholesterolemic effect of *Bacopa monniera* linn. on high cholesterol diet induced hypercholesterolemia in rats, *Asian Pacific Journal of Tropical Medicine*, 2012, 949-955.

90   V Madhavan, Ranajit Damodhar Tijare[1], R Mythreyi, M R Gurudeva and S N Yoganarasimhan, Pharmacognostical studies on the root tubers of *Asparagus gonoclados* Baker- Alternate source for the ayurvedic drug Shatavari, *Indian Journal of Natural Products and Resources* Vol. 1 (1), 2010, pp 57-62.

91   Velavan S, Nagulendran KR, Mahesh R and Hazeena Begum, The chemistry, Pharmacological and Therapeutic Applications of *Asparagus racemosus*- A Review, *Phcog Rev*, 2007, 1(2), 350-360.

92   Visavadiya NP and Narasimhacharya RL, Hypolipidemic and antioxidant activities in *Asparagus racemosus* in hypercholesteremic rats, *Indian J Pharmacol*, 2005, 37, 376-380.

93   Wiart C, Etnopharmacology of medicinal plants, New Jersey: Humana Press; 2006, pp: 1–50.

94   Xin Liu, Zhenliang Sun, Miansong Zhang, Xiumei Meng, Xuekui Xia, Wenpeng Yuan, Feng Xue, Changheng Liu, Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber Apostichopus japonicas, *Carbohydrate polymers 90,* 2012, 1664-1670.

95   Yoganarasimhan SN, Medicinal plants of India, vol. 2-Tamil nadu, cyber Media, Bangalore, 2000, p. 61.

96   Web References:

97   http://www.enzim.hu/~lbarna/articles/11820796.pdf

98   www.iloveindia.com, *Indian Herbs*

99   "About cholesterol" (http:/ / www. americanheart. org/ cholesterol/ about. jsp) – American Heart Association**.**

100   Satyanarayana U, Biochemistry, 2nd edition, Vijayawada: Uppala author-publishers interlinks; 2002.

101   Jerry Gordon, How cholesterol works, Free newsletter, Available from URL: *http://science.how* stuff works.com//cholesterol.htm

102   .

103   Goodman and Gilmans. The pharmacological basis of therapeutics. 10th ed. New York. Macgraw hill medical publishing division: 2001.

104   American heart association, cholesterol, Available from URL: *http://www.americanheart.org/presenter.jhtml?identifier=4600.Accessed on Nov. 25.2005.*