“A STUDY TO EXPLORE THE WEIGHT REDUCING PROPERTY OF *DOLICHOS BIFLORUS* AND METFORMIN USING OBESE RAT MODEL - MECHANISTIC STUDY”

DISSERTATION

SUBMITTED FOR

M.D. PHARMACOLOGY

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DEPARTMENT OF PHARMACOLOGY

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PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

COIMBATORE

CERTIFICATE

This is to certify that this dissertation entitled **"A study to explore the weight reducing property of *Dolichos Biflorus* and Metformin using obese rat model- mechanistic study"** by **Dr. P. Mary Mala,** is a work done by her during the period of study in the Department of Pharmacology from June 2016 to May 2019, under the guidance of **Dr.K.Bhuvaneswari, M.D.PGDBE,** Professor & HOD, Department of Pharmacology, PSG IMS&R.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled, **"A study to explore the weight reducing property of *Dolichos Biflorus* and Metformin using obese rat model- mechanistic study",** is a bonafide work done by me under the guidance and supervision of Dr.K.Bhuvaneswari, HOD & Professor, Department of Pharmacology, PSG Institute of Medical Sciences & Research. This study was conducted at the PSG Institute of Medical Sciences & Research, Coimbatore, under the aegis of The Tamilnadu Dr.MGR Medical University, Chennai, as part of the requirement for the award of M.D. Degree in Pharmacology.

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DATE: 21.11.2016

Title of the Project: To explore the weight reducing property of DOLICHOS BIFLORUS and metformin using obese rat model-Mechanistic study.

Proposal Number:	341/2016/ IAEC
Name of the Applicant:	Dr.Mary mala
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Methodology:	Approved

Name of species: **Swiss albino mice/ Wistar rats/ Sprague Dawley rats/ Guinea pigs/ Newzealand White rabbits.**
18 male albino rats

Male/Female/Both sex----------animals approved.

Signature of Chairperson

Date: 21.11.16

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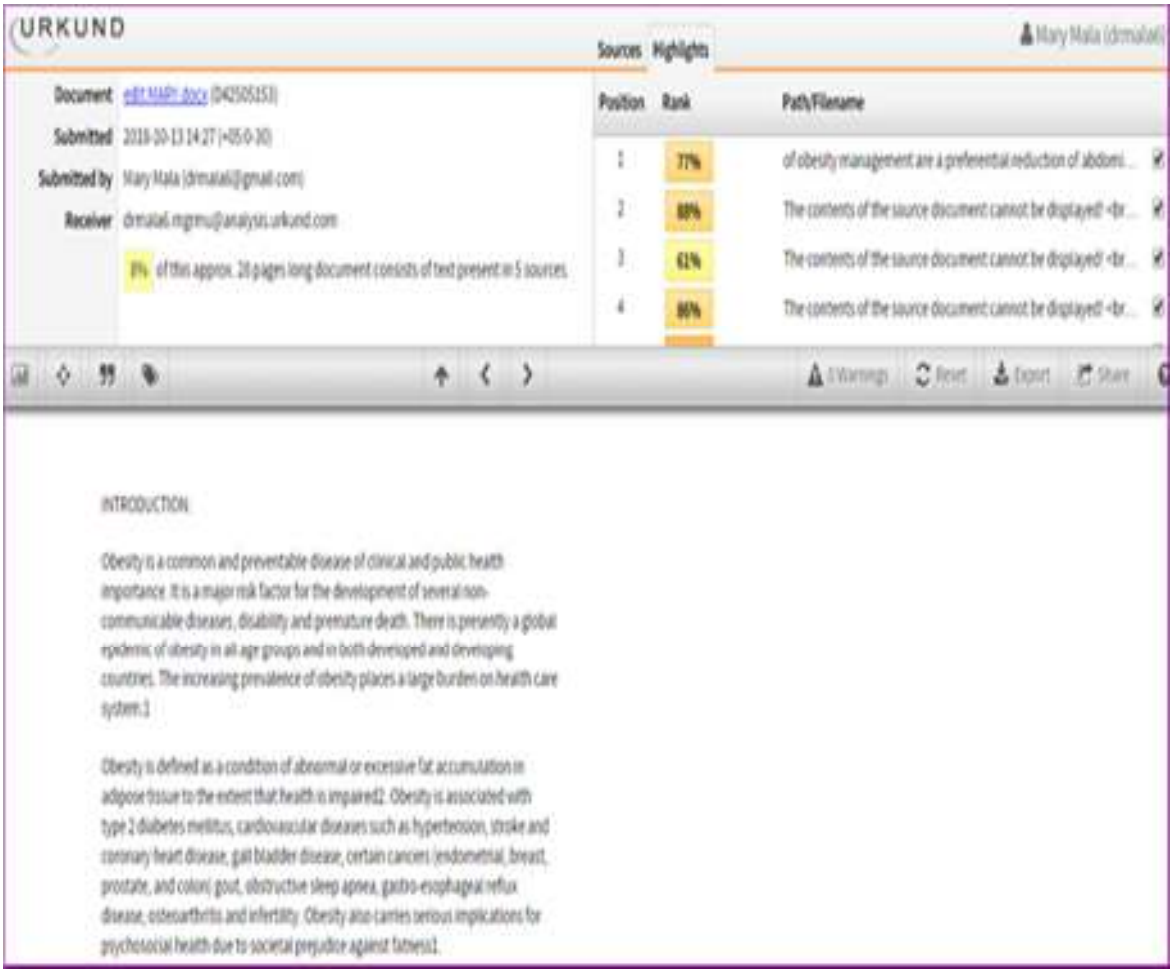
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INTRODUCTION
Obesity is a common and preventable disease of clinical and public health importance. It is a major risk factor for the development of several non-communicable diseases, disability and premature death. There is presently a global epidemic of obesity in all age groups and in both developed and developing countries. The increasing prevalence of obesity places a large burden on health care system.1
Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue to the extent that health is impaired2. Obesity is associated with type 2 diabetes mellitus, cardiovascular diseases such as hypertension, stroke and coronary heart disease, gall bladder disease, certain cancers (endometrial, breast, prostate, and colon), gout, obstructive sleep apnea, gastro-esophageal reflux disease, osteoarthritis and infertility. Obesity also carries serious implications for psychosocial health due to societal prejudice against fatness1.

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Dr.Mary Mala.P

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INTRODUCTION

Obesity is a common and preventable disease of clinical and public health importance. It is a major risk factor for the development of several non-communicable diseases, disability and premature death. There is presently a global epidemic of obesity in all age groups and in both developed and developing countries. The increasing prevalence of obesity places a large burden on health care system.[1]

Obesity is defined as a condition of abnormal or excessive fat accumulation in adipose tissue to the extent that health is impaired[2]. Obesity is associated with type 2 diabetes mellitus, cardiovascular diseases such as hypertension, stroke and coronary heart disease, gall bladder disease, certain cancers (endometrial, breast, prostate, and colon) gout, obstructive sleep apnea, gastro-esophageal reflux disease, osteoarthritis and infertility. Obesity also carries serious implications for psychosocial health due to societal prejudice against fatness[1].

The global epidemic of obesity has resulted mainly from societal factors that promote sedentary lifestyles and the consumption of high-fat, energy-dense diets. While genes are important in the determination of a person's susceptibility to weight gain, obesity occurs when energy intake exceeds energy expenditure over a prolonged period. Obese children are more prone to become obese adults than normal children[1]

Etiology of obesity is multifactorial, involving complex interactions among genetic factors, hormones, social and environmental factors. Nutrition transition as a result of urbanization and affluence has been considered as the

major cause for obesity epidemic. Major dietary changes include a higher energy density diet with a greater proportion for fat and added sugars in foods, greater saturated fat intake, marked increase in animal food consumption, reduced intake of complex carbohydrates and dietary fiber and also reduced intake of fruits and vegetables. Several studies have shown that insufficient physical activity is one of the important risk factors of obesity. Work-related activity has declined over recent decades in industrialized countries whereas leisure time dominated by television viewing and other physically inactive pastimes has increased[3].

The effective treatment of obesity should address both the medical and social burden of this disease. Goals of obesity treatment include a preferential reduction of abdominal fat, amelioration of obesity related health risks, an improvement in co morbidities and quality of life and a reduction in mortality rate[4]. Low-calorie low-fat diet, increased physical activity and lifestyle modification are essential for the treatment of obesity. A low-energy diet recommended for the treatment of obesity should be low fat (<30%), high carbohydrate (55% of daily energy intake), high protein (up to 25% of daily energy intake) and high fiber (25g/day). Physical activity should be an integral part of the comprehensive obesity management. Behavioral management includes several techniques such as self-monitoring, stress management, stimulus control, reinforcement techniques, problem solving, rewarding changes in behavior, cognitive restructuring, social support and relapse prevention training.

Antiobesity drugs assist weight loss in combination with lifestyle management, to improve weight loss maintenance and to reduce obesity-related

health risks. Anti-obesity drugs affect different targets in the central nervous system or peripheral tissues and aim to normalize or regulate metabolic disturbances that are involved in the pathogenesis of obesity. Weight loss induced by currently available anti-obesity drugs is only modest, reaching usually 5–8% of initial body weight. Bariatric surgery is the most effective treatment for morbid obesity in terms of weight loss and improvement in quality of life. It should be considered for patients with BMI $\geq$40.0 kg/m^2 or with BMI between 35.0 and 39.9 kg/m^2 with co morbidities. Studies show that only 5-10% subjects can maintain their weight loss over the years.

Diet and exercise are best for both prevention and treatment but require much discipline and are difficult to maintain. Medications offer a possible adjunct, but their effect is modest, they are limited by side effects and weight loss lasts only as long as the drug is being taken, since as soon as treatment is stopped, weight is regained. Pharmaco therapy is offered to those who have failed to achieve weight loss goals through diet and exercise alone[5].

Currently approved drugs for pharmacotherapy of obesity are Orlistat, Locarserin, Fluoxetine, Phentermine/Topiramate and Naltrexone/Bupropion combinations. Orlistat (Xenical) is a lipase inhibitor. It reduces dietary fat absorption by 30% by inhibiting pancreatic and gastric lipase. The side effects of Orlistat therapy are intestinal cramps, flatus, fecal incontinence, oily spotting and discharge. Levels of fat-soluble vitamins (A, D, E) and beta-carotene are lowered by Orlistat therapy.

GLP-1 is a hormone secreted by L cells in the terminal ileum after food intake. It decreases blood glucose by inhibiting glucagon secretion and stimulating insulin secretion. GLP-1 also delays gastric emptying, reduces

calore intake and promotes satiety. Exenatide (Byetta) is a GLP-1 receptor agonist and has a much longer half life because it is resistant to dipeptidyl peptidase-4 mediated degradation. It is approved to treat type 2 diabetes and produces similar effects to GLP-1, reducing fasting and postprandial blood glucose levels, decreasing haemoglobin A1c, slowing gastric emptying and decreasing food intake .In diabetic patients it has been shown to cause weight loss by an average of 1.6kg without any change in lifestyle, diet or exercise. Nausea is a common adverse effect. Acute pancreatitis can occur in patients taking Exenatide. Exenatide is available in prefilled syringes is administered subcutaneously twice daily immediately before or within one hour of morning and evening meals. Exenatide should not be used in patients with severe renal impairment or end-stage kidney disease

Liraglutide (Victoza) is a long-acting GLP-1 analog. In diabetes trials, Liraglutide was associated with a significant reduction in weight (2.0 to 2.5kg) when compared with placebo or Glimepiride. Liraglutide is available in prefilled pens. The initial dose is 0.6mg once daily for one week .After one week, the dose should be increased to 1.2mg once daily for one week. If blood glucoses remain above the goal range, the dose can be increased to 1.8mg once daily.

Pramlintide (Symlin) is an analogue of amylin, a small peptide hormone that is released into the bloodstream by the β-cells of the pancreas along with insulin in response to nutrient stimuli. Amylin has been shown to slow gastric emptying, reduce postprandial rise in blood glucose concentration and improve hemoglobin A1C concentrations in both type 1 and type 2 diabetes patients. It

is given by subcutaneous injection, at a maximum dose of 120 μ micrograms with each meal.

Topiramate (Topamax) is an anticonvulsant agent approved for the treatment of refractory seizures. It mitigated weight gain observed with antidepressant treatment. Topiramate is associated with secondary acute angle glaucoma, decrease in serum bicarbonate and development of metabolic acidosis, low urinary citrate excretion and increased urinary pH.

Bupropion is a Norepinephrine and Dopamine reuptake inhibitor that is approved for treating depression and smoking cessation. Bupropion seems to have a weight-neutral effect for most depressed individuals of normal weight. Controlled trials with obese depressed individuals suggest that bupropion SR may be associated with weight loss in obese subjects. Weight gain after treatment for smoking cessation was less in bupropion SR-treated subjects than in placebo treated subjects.

Serotonin and agonists that activate serotonin 2C receptors promote feelings of satiety, thereby reducing food intake. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) approved for the treatment of depression may also lead to weight loss. Stimulation of specific central serotonin receptors is an effective pharmacological mechanism to suppress appetite. Lorcaserin is a selective serotonin 2C agonist for treating obesity.

Fenfluramine and Phentermine was withdrawn after it was reported that it could cause valvular heart disease and pulmonary hypertension. Phenylpropanolamine was withdrawn as it was to found to be an independent risk factor for hemorrhagic stroke in women. Rimonabant, the selective cannabinoid (CB1) receptor blocker was withdrawn because it caused

increased depression and suicidal ideation. Sibutramine (Meridia, Reductil) was first developed as an antidepressant. It is a centrally acting Noradrenaline and serotonin reuptake inhibitor that enhances weight loss by increasing satiety. The drug was withdrawn due to cardiovascular side effects.

Several new targets are currently being evaluated such as amylin analogues (Davalintide), leptin analogues (Metreleptin), MC4R agonists (RM-493), oxyntomodulin analogues, neuropeptideY antagonists (Velneperit), cannabinoid type-1 receptor blockers (AM-6545), lipase inhibitors (Cetilistat) and anti-obesity vaccines (ghrelin, somatostatin).[6]

Disappointing results after lifestyle modification or pharmacotherapy indicated the need for other treatment modalities to produce better and long-lasting results in terms of weight loss. Herbal supplements and diet-based therapies for weight loss are among the most common complementary and alternative treatment modalities .A vast range of these natural products and medicinal plants, including crude extracts and isolated compounds from plants can be used to induce weight loss and prevent diet-induced obesity. Nigella sativa, Camellia sinensis, green tea and black Chinese tea were found to have acceptable anti-obesity effects.[7]

Metformin which is most widely used for the treatment of type 2 diabetes mellitus may be useful in aiding weight loss. In diabetic patients, it suppresses endogenous glucose production and also acts as an insulin sensitizer. It also helps diabetic patients lose weight or at least keep their weight stable[8, 9].

The mechanism of weight loss due to Metformin in diabetic patients is attributed to decreased food intake and reduction in calorie intake.[10]

In PCOS women with abdominal obesity, long-term treatment with Metformin, added to hypocaloric diet, induced a greater reduction of body weight and visceral fat and a more consistent decrease of serum insulin and leptin concentration[11].

Dolichos biflorus, commonly known as horse gram as it was used widely to feed horses. However, in Tamil nadu it is used to make dishes. In traditional Siddha cuisine, horse gram is considered a food with medicinal qualities. This pulse is a demulcent to reduce cough. It is also used to reduce corpulence. There is even a Tamil proverb which says: “Ilaiththavanukku ellu; Kozhuththavanuuku kollu”.[12]

This is a mechanistic study to explore the mechanism of weight reducing property of Dolichos biflorus and Metformin using obese rat model.

AIM & OBJECTIVES

PRIMARY OBJECTIVE:

To find out the role of cold extract of dolichos biflorus in preventing and treating obesity in high fat diet induced obese rat model.

SECONDARY OBJECTIVE:

To understand the mechanism of weight reducing property of cold extract of Dolichos biflorus in preventing and treating obesity in comparison with Metformin.

1. Central mechanism
2. Antioxidant property
3. Anti-inflammatory property
4. Metabolic effects

LITERATURE REVIEW

Obesity is defined as abnormal or excessive fat accumulation that may impair health. WHO defines overweight as BMI equal to or more than 25 and obesity as BMI equal to or more than 30.[13]

PATHOPHYSIOLOGY OF OBESITY:

Obesity is a disorder of energy regulation.

Regulation of energy balance is complex and has three components.

1. Afferent signals are Insulin, Ghrelin, Leptin, Peptide YY.

2. Hypothalamus integrates afferent signals and triggers efferent signals.

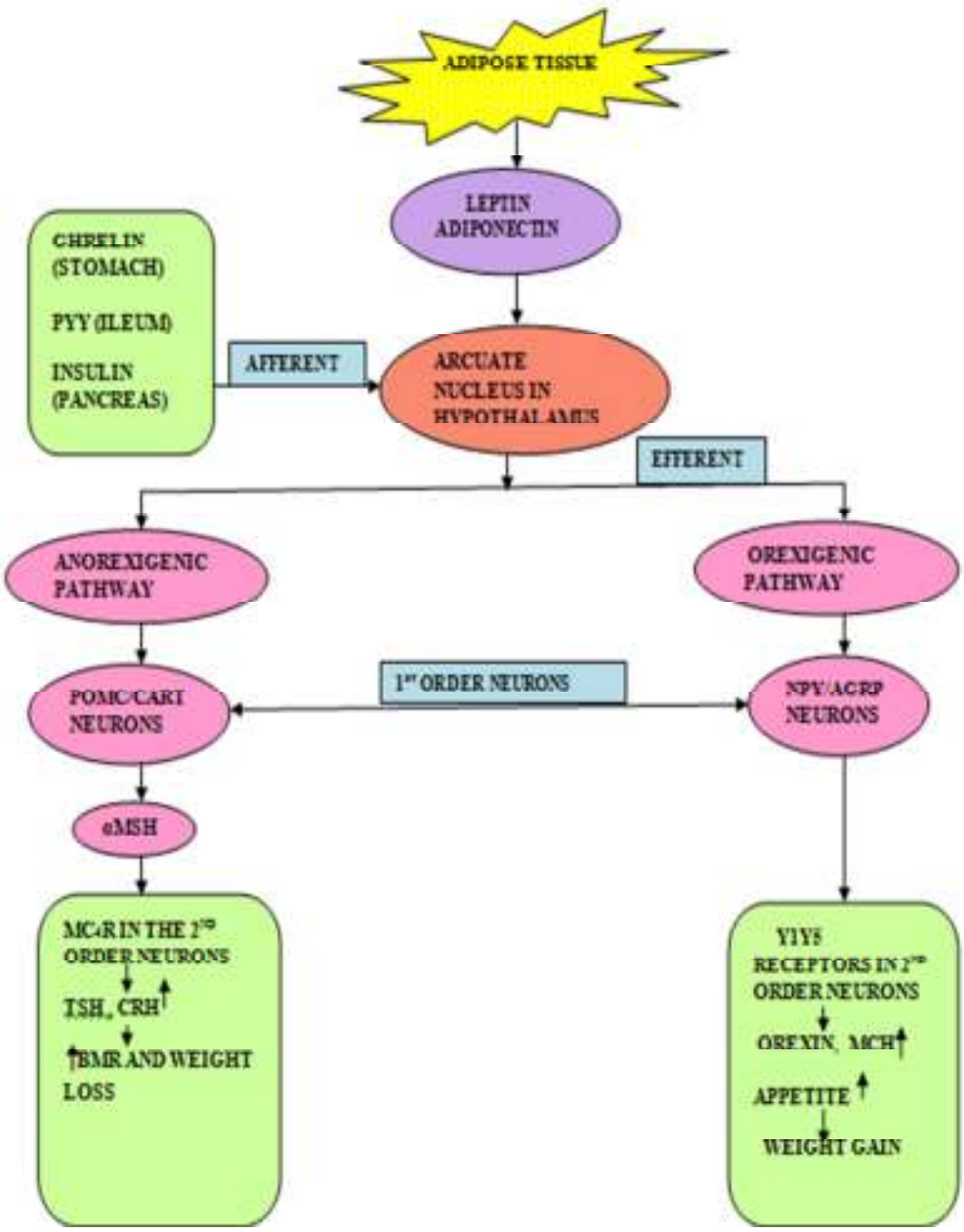
3. Efferent signals which controls energy balance.

The peripheral or afferent system generates signals from various sites. Its main components are Leptin and Adiponectin produced by fat cells, Ghrelin from the stomach, Peptide YY from the ileum and colon and Insulin from pancreas. The Arcuate nucleus in the Hypothalamus processes and integrates neurohumoral peripheral signals and generates efferent signals. The first order neurons in the hypothalamus are Proopiomelanocortin (POMC) and Cocaine and Amphetamine regulated transcript (CART) and Neurons containing neuropeptide Y (NPY) and Agouti related peptide (AgRP). These first order neurons communicate with second order neurons in the hypothalamus. The efferent system is organized along two pathways anabolic and catabolic that control food intake and energy expenditure respectively. The hypothalamus also communicates with forebrain and midbrain centers that control the autonomic nervous system. POMC /CART neurons enhance energy

expenditure and weight loss through the production of α melanocyte stimulating hormone and the activation of melanocortin receptors 3 and 4 (MC3/4R)in second order neurons .These second order neurons in turn produce Thyroid stimulating hormone, Corticotropin releasing hormone(CRH) that increase BMR and anabolic metabolism leading to weight loss. NPY/AgRP neurons promote food intake (orexigenic effect) and weight gain through the activation of Y1/5 receptors in secondary neurons. These secondary neurons then release melanin concentrating hormone (MCH) and orexin which stimulates appetite.

Many peripheral hormones participate in central nervous system control of appetite, food intake, food reward or addiction. Hunger and satiety signals from adipose tissue (leptin), the pancreas (insulin) and the gastrointestinal tract (CCK), glucagon-like peptide-l (GLP-1), peptide YY3-36 and ghrelin are involved in relaying information about energy status through the neural hormonal gut-brain axis primarily targeting the hypothalamus and brainstem and may directly or indirectly interact with the midbrain dopamine pathways to impact feeding[14-17] .

FIGURE 1: PATHOPHYSIOLOGY OF OBESIY



OBESITY AND COMORBIDITIES:

DIABETES:

Obesity is the leading risk factor for type 2 diabetes. Three mechanisms have been proposed to link obesity to insulin resistance and predispose to type 2 diabetes: 1) increased production of adipokines/cytokines including tumor necrosis factor-α, resistin and retinol-binding protein 4 that contribute to insulin resistance, reduced levels of adiponectin 2) ectopic fat deposition, particularly in the liver and in skeletal muscle 3) mitochondrial dysfunction[18-20]

OBESITY AND INSULIN RESISTANCE:

Obesity is related to insulin resistance and type 2 diabetes. Insulin resistance is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output.

Adipocytes function as endocrine glands with wide-reaching effects on other organs including brain by releasing hormones such as leptin, cytokines such as TNF-α and substrates such as FFAs. Thus adipose tissue play a major regulatory role in energy balance and glucose homeostasis. [21-23]

OBESITY AND HYPERTENSION:

Activation of the sympathetic nervous system (SNS), has been considered to have a crucial function in the pathogenesis of hypertension among obese individuals. The mechanisms that have been proposed to be responsible for an increased sympathetic activity in obesity include impaired function of the baroreceptor sensitivity, increased levels of circulating free-

fatty acids (FFAs), plasma renin, angiotensin II, insulin, aldosterone and leptin. Obesity represents a state of inflammation (vascular and systemic) that can cause endothelial dysfunction.[24-25]

OBSTRUCTIVE SLEEP APNEA (OSA):

OSA is a common disorder whose prevalence is linked to an epidemic of obesity in western society. Sleep apnea is due to recurrent episodes of upper airway obstruction that is caused by elevation in upper airway collapsibility during sleep. Obesity is a potent risk factor for the development and progression of sleep apnea. With increasing obesity, sleep apnea can contribute to the development of daytime alveolar hypoventilation (obesity hypoventilation syndrome), cor pulmonale and frank respiratory failure.[26-27]

OBESITY AND INFERTILITY:

Infertility affects one in seven couples and its rate is on the increase. Ovulatory defects and unexplained causes account for >50% of infertility aetiology. It is postulated that a significant proportion of these cases are either directly or indirectly related to obesity.

Overweight women have a higher incidence of menstrual dysfunction and anovulation. The risk of sub fecundity and infertility, conception rates, miscarriage rates, and pregnancy complications are increased in these women. They have poor reproductive outcomes in natural as well as assisted conception. Obesity affects the HPG axis by increased free estrogen levels due to increased conversion of androgens to estrogens in adipose tissue. Increased estrogen causes a decrease in GnRH by negative feedback thus leading to

irregular or anovulatory cycles. Anovulation is due to hyperinsulinemia, insulin resistance and hyperandrogenism. Up to 35%–60% of patients with PCOS are obese and menstrual disturbances, anovulation and infertility are more common in these obese PCOS patients than in non-obese PCOS patients[28-29].

OBESITY AND CANCER

Recent research has found that as the body mass index increases by 5 kg/m^2, cancer mortality increases by 10%.The link between obesity and cancer has been demonstrated in numerous cohort studies. The International Agency for Research on Cancer (IARC) concluded that there is adequate evidence of an association between obesity and several cancers, including colon, postmenopausal breast, endometrial, renal and esophageal cancers. The percentage of cancer attributed to obesity was 11% for colon cancer, 9% for postmenopausal breast cancer, 39% for endometrial cancer, 25% for renal cancer and 37% for esophageal cancer. Insulin and insulin-like growth factor 1 (IGF-1) are pathways that are linked to obesity and work to prohibit apoptosis and promote cell proliferation. It is hypothesized that the increased level of insulin reduces the amount of IGFBPs, which leads to an increase in the level of IGF-1 and a change in cell environment which promotes tumor growth. Sex steroid hormones like estrogens (E1-estrone and E2-estradiol), androgens and progestogens are mainly produced by the adrenal glands. Estrogen binds to the receptor (ER), activating the intracellular signaling pathways that will initiate tumor progression by stimulating cell division. Excess aromatase from the adipose tissue can lead to higher levels of estradiol that are not bound causing

further DNA damage. Estrogens also interact with IGF, which promotes tumor growth through inhibition of apoptosis. Adipokines, hormones produced from adipose tissue also have been proposed as a possible link between obesity and cancer. Colon, prostate, and breast cancers have been associated with increased serum leptin levels. Obesity may decrease the antioxidant activity and induce oxidative stress thus increasing the cancer risk[30-31].

OBESITY AND CARDIOVASCULAR DISEASE:

Under normal physiological circumstances, adipocytes release anti-inflammatory factors such as adiponectin, transforming growth factor-beta, interleukin-10 and nitric oxide, which promote insulin-sensitivity and anti-atherogenic effects. In contrast, pathologic hypertrophied adipocytes caused by excessive body weight release pro-inflammatory cytokines such as leptin, tumour necrosis factor-alpha, resistin and interleukin-6 contributing to the development of various metabolic diseases. Adiponectin which is an antidiabetogenic and antiatherosclerotic adipokine is found in high levels in the blood of lean healthy individuals whereas its concentration is markedly reduced among individuals with type 2 diabetes, coronary heart disease (CHD) or with overweight [32-33].

OBESITY AND OSTEOARTHRITIS (OA):

Obesity is the greatest modifiable risk factor for osteoarthrits Coggon *et al* reported that subjects with a BMI>30 kg/m^2 were 6.8 times more likely to develop OA knee than normal-weight controls. Structural joint damage is thought to result from mechanical factors, decreased muscle strength, altered

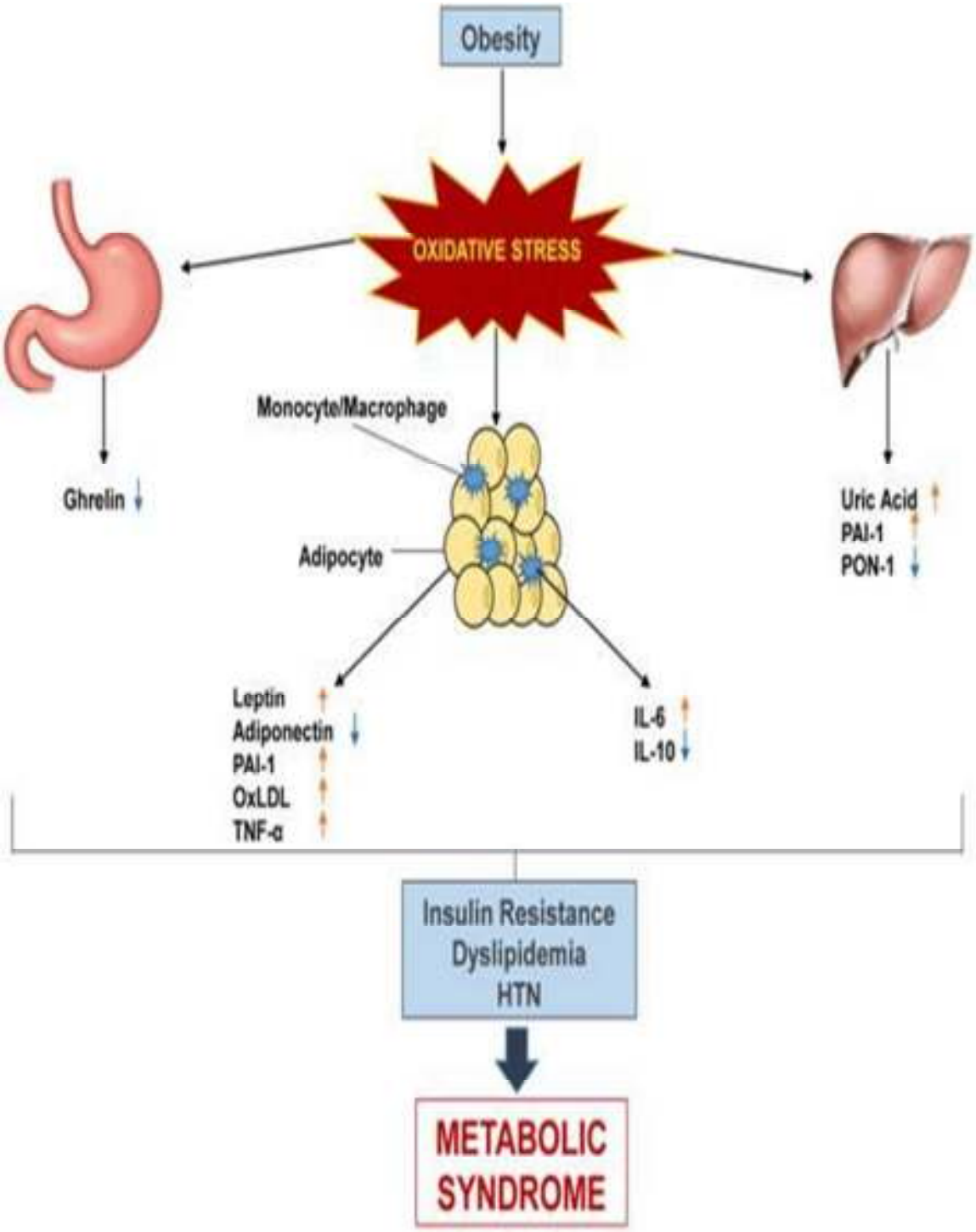
biomechanics during everyday activities and metabolic factors. The metabolic pathways through which obesity contributes to joint structural damage involve aberrant adipokine expression with direct and downstream effects leading to destruction and remodeling of joint tissue.[34]

OBESITY AND METABOLIC SYNDROME:

Obesity is one of the components of metabolic syndrome. Oxidative stress, the hallmark of obesity, is linked to a chronic low-grade inflammation. Systemic oxidative stress promotes inflammation, results in endothelial dysfunction and altered lipid metabolism and affects insulin sensitivity.

Leptin, IL-6, TNF-α, have been shown to be elevated in metabolic syndrome. On the other hand, adiponectin, ghrelin, IL-10 have shown to be decreased in metabolic syndrome[35]

IMAGE 1: METABOLIC SYNDROME & OBESITY [35]



DOLICHOS BIFLORUS:

Medicinal plants have been used in traditional medicine as they have immune potential against various diseases. Dolichos biflorus is a well known medicinal plant in folklore for its medicinal properties. In herbal medicine the seeds are mainly used as tonic, astringent, diuretic and also recommended in asthma, bronchitis, urinary discharges, hiccoughs, ozoena, heart trouble and other diseases of brain. Seeds extract of Dolichos biflorus has exhibited mild analgesic and diuretic activity[36].

Dolichos biflorus is known for its lipid lowering property. Hypolipidemic effect is screened using wistar albino rats and effects are compared with that of tab Atorvastatin. Treatment with methanolic extract of dolichos decreased free fatty acid concentration, VLDL and LDL levels significantly. HDL concentration in plasma increased after treatment with dolichos.[37-38]

Anti-inflammatory effects of various doses of methanolic extract of dolichos (DME) were evaluated in acute inflammatory model induced by carrageenan in albino rats. Carrageenan-induced rats showed an increased activity of COX, whereas DME administration significantly decreased the COX activity. Based on these reports it is inferred that the inhibitory effect of DME (50 mg/kg body weight) on carrageenan-induced inflammation in rats may be due to inhibition of the enzyme COX leading to inhibition of prostaglandin synthesis. The level of NOS in the monocytes was significantly increased in the carrageenan-induced groups, whereas it was decreased in the

DME-treated group. The increased concentration of lipid peroxidation product, measured as malondialdehyde (MDA) is used to assess the extent of inflammation .The increased levels of MDA in the carrageenan-induced rats indicate increased lipid peroxidation during inflammation that contributes to the increased swelling of the paw. On treatment with DME, a significant decrease in MDA levels were observed, which indicate the efficiency of DME in inhibiting lipid peroxidation. The endogenous antioxidants that can scavenge Reactive Oxygen Species (ROS) are catalase and superoxide dismutase (SOD). The DME treatment significantly enhanced the catalase activity thus protecting the cell from oxidative damage. The carrageenan induced rats showed a decrease in the activity of catalase. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense. DME administration significantly increased the activity of SOD compared to carrageenan-induced rats. The effect of DME was more pronounced than the standard drug Voveran. Vitamin C is a strong antioxidant and its level was increased upon DME treatment compared to the carrageenan-induced rat.[39]

In order to develop an effective and safe weight loss ingredient extracts of more than four hundred medicinal plants were screened for their ability to inhibit adiposity in 3T3-L1 mouse adipocytes. Among the candidate plant extracts tested, Piper betel leaf extract and Dolichos biflorus seed extract showed potent anti-adipogenic efficacy. Combination of Piper betel leaf extract and Dolicho*s* biflorus seed extract in a ratio of 2:3 also known as LOWAT

demonstrated greater anti-adipogenic and lipolytic activities compared to the individual extracts.[40]

In another study hot extract of horse gram was prepared and was given to male and female obese human volunteers. Daily monitoring of body weight prior to horse gram extract administration done to ensure correct dosing of horse gram extract every day. Weekly assessment of % gain or loss of body weight was calculated based on pretreatment body weight for each volunteer. It was found that hot extract of horse gram has antiobesity activity. The onset of anti obesity effect was quicker in males than females[41].

In another study healthy adult albino rats of wistar strain were housed in standard polypropylene cages and fed with standard pellet diet and water ad libitum. The animals were divided into 3 groups. Group1 was given hot extract at a dose of 4% w/v in drinking water. Group2 was given cold extract 4% w/v in drinking water. Control group was given drinking water. Freshly prepared extracts were given daily for a period of 4 weeks. Body weight, 24 hours food intake, behavior satiety sequence were analyzed. All the animals in the 3 groups showed an increase in body weight over a period of 4 weeks. The cold extract attenuated the rise in body weight since there was no difference between the mean body weight at baseline and that obtained after treatment. Thus it was proved that cold extract of Dolichos biflorus seeds possesses anorectic activity.[42]

ANIMAL MODELS OF OBESITY:

SURGICALLY INDUCED HYPOTHALAMIC OBESITY:

Hypothalamus regulates food intake by interaction of a lateral feeding centre and a medial satiety centre. Injury to hypothalamus can be produced by surgical methods. Female sprague dawley rats are fed high fat diet for 5 to 9 days. After this rats are anaesthetized with 35 mg/kg pentobarbital sodium along with 1 mg atropine methyl nitrate given intraperitoneally. Bilateral knife cuts are stereotactically made in the hypothalamus of rats. Sham operated rats serve as control. Food intake, body weights are recorded and compared.

CHEMICALLY INDUCED HYPOTHALAMIC OBESITY:

1. **Mono glutamate induced hypothalamic obesity in mice:**

Mice are injected daily with monosodium glutamate subcutaneously in the dose of 2 g/ kg for 5 consecutive days .In control mice physiological saline is injected. All mice are house under maintained temperature and artificial light dark cycle and provided with chow and water ad libitum. Food consumption and weight are recorded and compared between the groups.

2. **Gold thioglucose induced hypothalamic obesity in mice**:

Six weeks old Swiss albino mice of either sex are used. Single dose of gold thioglucose 30 to 40 mg/ kg is given by intraperitoneal route. Body weight is recorded for 3 months and compared with normal control.

VIRUS INDUCED OBESITY:

Mice infected with canine distemper virus develop obesity after 8 to 10 weeks of viral infection. Canine distemper virus targets hypothalamus and

causes disruption of critical brain catecholamine pathways as a result of which obesity is developed. Other viruses that can cause obesity in animals are borna disease virus and avian adenovirus.

GENETIC MODELS OF OBESITY:

Genetic models may be monogenic and polygenic**.**

1. Monogenic models of obesity:

Yellow obese mouse, Obese (ob/ob) mouse, Diabetes (db/db) mouse, fat mouse, Tubby mouse, Obese SHR rats

2. Polygenic models of obesity:

Japanese KK mouse, NZO mouse, M16 mouse, OLEFT rats

TRANSGENIC ANIMAL MODELS:

In these animal models genes regulating energy homeostasis are manipulated**.**

HIGH FAT INDUCED OBESITY:

Exposure of animals to high fat diets results in the development of obesity. The caloric density of high fat diets and the resulting higher intake of total energy contribute to this effect. High fat diets rapidly and specifically reduce the central actions of insulin and leptin, most likely due to a post-receptor effect. This effect is rapid, occurring after a few days of high fat exposure. High fat diets directly affect the respective intracellular signaling pathways in hypothalamic target neurons with resulting changes in neuropeptide expression. Fat composition seems to have a major role in this effect because saturated fat is more deleterious than unsaturated fat[43],[44].

High fat diet increases energy intake, weight, body fat mass, mesenteric adipocytes size and plasma leptin levels and decreases oral glucose tolerance in both Wistar and Sprague Dawley rat (SD). Wistar rats fed with High fat diet consumed higher amounts of food and therefore higher amounts of energy when compared to SD rats fed with the same diet. Consequently, weight gain was larger in these animals and was mainly due to an expansion of adipose tissue mass. Leptin is an adipocyte-derived hormone that controls food intake and energy expenditure. Plasma leptin concentration increases in proportion to body fat mass As a result, Wistar rats fed with high fat diet displayed higher plasma leptin levels than SD rats in the same diet regimen. The amount of leptin released by each gram of body fat mass (plasma leptin to body fat mass ratio) was also more elevated in Wistar than in SD rats. Oral glucose tolerance was decreased in animals of both strains fed with high fat diet. Adiponectin is recognized by its insulin sensitizing action, obesity may induce a malfunction on adiponectin signaling. Hypertriglyceridemia is one of the criteria for diagnosis of the metabolic syndrome and seems to be present in Wistar rats fed with high fat diet.[45]

High fat diet induced obesity shares many features with human obesity. High fat rats weighed more than low fat controls. They developed more adipose tissue than control rats and acquired insulin resistance and hyperleptinemia typically associated with obesity. This model is therefore well suited for a systematic investigation of the role of dietary fat on body weight regulation and can be applied to many questions that are central to obesity

research. The obesity was manifested as a modest but significant 10% increase in total body weight. Rats consuming the high fat diet gained increased body fat relative to rats eating either of two quite different low fat diets. The model described shares many important features with human obesity. The phenomenon is reliable and consistent from one experiment to the next. This validates making comparisons across experiments when the same diets and procedures are used to produce the obese state. The obese rats are both hyperleptinemic and hyperinsulinemic and as occurs in humans, both plasma insulin and leptin concentrations were directly correlated with the degree of adiposity. Hyperinsulinemia in the high fat rats was a result of insulin resistance, a common feature of human obesity and is central to the development of diabetes and cardiovascular disease. The explanation for the ability of the high fat diet to induce obesity is overconsumption of this diet rather than specific metabolic effects of differing proportions of fat and carbohydrate in the diet[46].

Epidemiological studies have shown a positive relationship between dietary fat intake and obesity. Since rats and mice show a similar relationship, they are considered an appropriate model for studying dietary obesity. Dietary fat intake has been claimed as responsible for increase in adiposity. Human studies have shown that high-fat diets (30 % of energy from fat) can easily induce obesity. Diets rich in fat not only induce obesity in humans but also make animals obese. In both rats and mice a positive relationship has been found between the level of fat in the diet and body weight or fat gain. High-fat

diets within the range of 30–78 % of total energy intake are used either by adding a particular fat to the animal's diet or using an assortment of fat and sugar rich supermarket foods (cafeteria diet) for studying obesity in rats and mice[47].

METFORMIN

Metformin is an anti-diabetic drug which reduces insulin resistance which is the underlying cause of both type 2 diabetes and PCOS. Metformin has been observed to cause weight loss in type 2 diabetes and nondiabetic obese patients. Improving insulin sensitivity may account for weight reduction under Metformin therapy. Metformin decreases appetite and certain authors discuss that Metformin contains a primary anorectic factor. One other reason may be a decrease of leptin levels.GLP-1 levels seem to rise significantly under Metformin therapy and may thus promote weight loss[48-51].

JUSTIFICATION:

Obesity is a major global health problem. Pharmacological agents available for treating obesity are associated with lot of adverse effects. Hence herbal preparations may be preferred over such drugs. Dolichos biflorus seed (kollu) is known for its ability to reduce weight in humans. Studies related to mechanism of weight reducing property of dolichos are not available. If this study hypothesis proves to be true this will provide a simple easily available household remedy for obesity. This may be used as an important pharmacological tool for the treatment of obesity in the future.

HYPOTHESIS:

The weight reducing property of dolichos biflorus may be due to central mechanism, anti-inflammatory effects, and antioxidant property.

Central mechanism	Eating behavior, Food intake, serum Leptin
Anti-inflammatory effect	Serum Adiponectin, serum lipase, serum interleukin6
Antioxidant property	Serum SOD, Serum MDA
Metabolic effects	Body weight, BMI, Serum cholesterol, Serum Triglycerides

MATERIALS & METHODS

After IAEC approval (341/2016/IHEC**)** 18 male Swiss albino rats were selected and were given food and water ad libitum and caged in physiological condition providing 12 hour: 12 hour dark and light cycle. Animals were divided into 3 groups of 6 animals each. 6 animals were taken as control and baseline parameters like weight, BMI, food intake analyzed. Blood samples were collected for analysis.

High Fat diets can be used to generate a valid rodent model for obesity. Obesity susceptible animals are hyperphagic, possibly due to a central resistance to the anorexigenic action of insulin and a decreased hypothalamic expression of anorexigenic peptides such as ∝ melanocyte stimulating hormone and cocaine and amphetamine-regulated transcript on an HF diet eat the same amount of calories as standard chow-fed controls. Prolonged feeding with fat-enriched diets induces an increase in body weight in susceptible rats in the range of 10% to 20% over standard chow-fed controls[52-54].

Obesity was induced by high fat diet for a period of 6 weeks. High fat diet (HFD) is prepared by mixing chow diet with butter 19 grams and soya bean oil 1 gram per 100 grams so that the fat content was 45%. Dolichos biflorus was known for its free radical scavenging activity and also has hypolipidemic property[55].

Dolichos seeds were purchased from the local farm and certified by the botanist. Dolichos cold extract (4%w/v) was prepared by soaking overnight 4 grams of dolichos seeds in 100 ml of water. Dolichos cold extract was prepared

fresh each day.[42]The effects of cold extracts of dolichos was compared with that of Metformin as Metformin improves peripheral and liver sensitivity to insulin, reduces basal liver glucose production, increases insulin-stimulated uptake and utilization of glucose by peripheral tissues, decreases appetite and causes weight reduction[56-58].

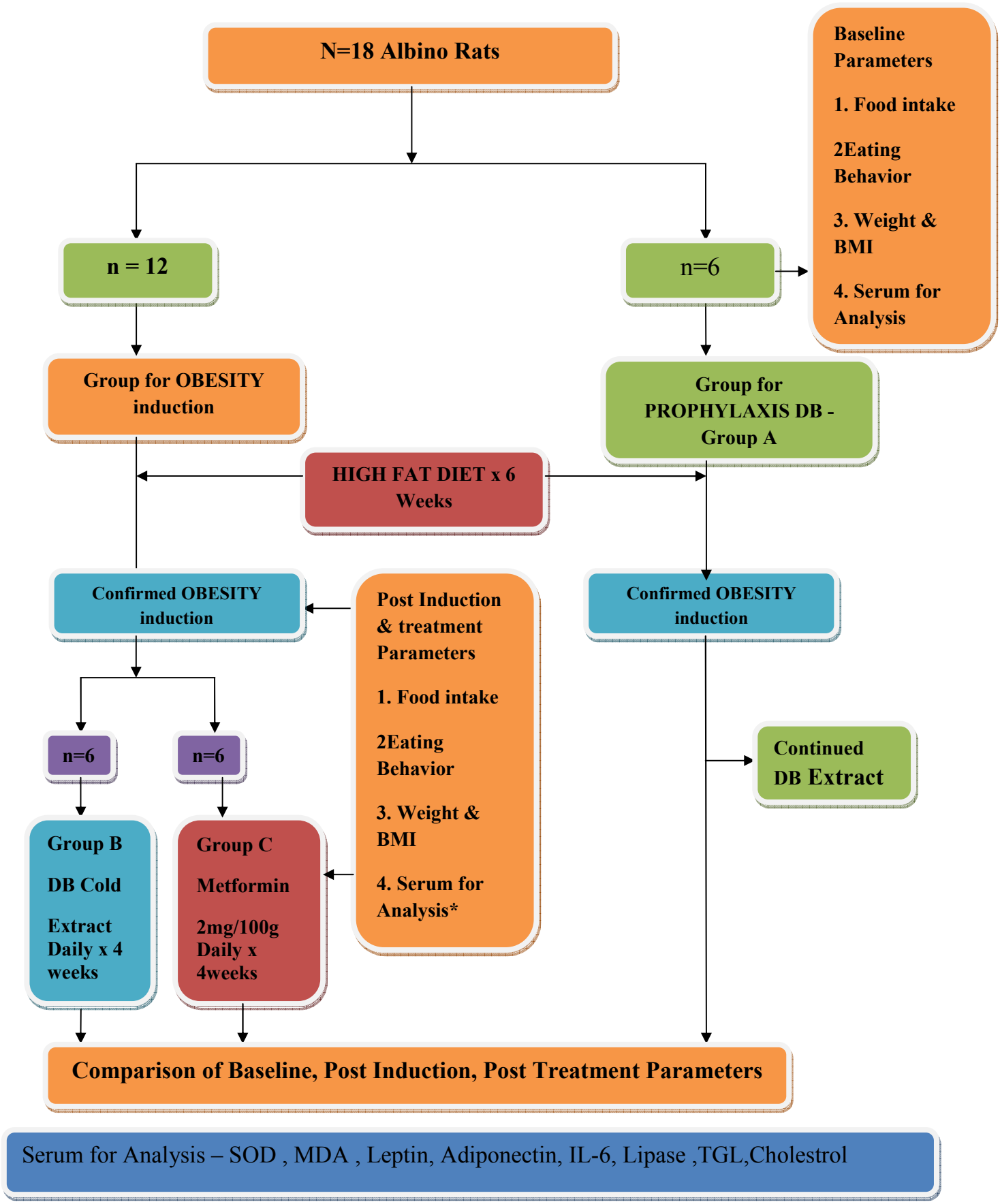
Group A was the prophylactic group in which cold extract of dolichos was given for a period of 6 weeks along with high fat diet and followed for a period of 4 weeks.

Group B was the dolichos group in which obesity was induced by high fat diet for a period of 6 weeks followed by treatment with cold extract of dolichos for 4 weeks

Group C was the Metformin group in which obesity was induced by high fat diet for 6 weeks followed by treatment with Metformin 2 mg per 100 g per day for a period of 4 weeks.[44,45]

Baseline, post induction and post treatment parameters were taken. Blood samples were taken for analysis.

FIGURE 2: STUDY DESIGN



BLOOD COLLECTION:

With the help of animal restrainer blood was collected from tail vein using standard method of blood collection under aseptic precautions. Bood was centrifuged and serum separated for analysis[59]

FOOD INTAKE:

Food intake was calculated for all groups of animals at baseline, post induction and after treatment using oxylet apparatus. Oxylet apparatus (Pan Lab Harvard Apparatus) has a physio cage. Animal was kept inside the cage. Calibration of feed and water intake was done. It was ensured standard atmospheric conditions were displayed. There should be no contact between cage and feed/water compartment. Measured quantity of food and drinking water was kept and food intake was calculated[42]

EATING BEHAVIOR

In rodents, grooming is a complex, phenomenon, which normally proceeds in a cephalocaudal direction and consists of several stages including licking the paws, washing movements over the head, fur licking, and tail/genitals cleaning. Grooming is highly sensitive to various stressors, psychotropic drugs and genetic manipulations. Animals were observed for one hour for activities like eating, grooming, rearing and resting at baseline, post induction and post treatment.[60]

CALCULATION OF BMI:

Weight of the animals was calculated at baseline, post induction and after treatment. Length of the animal was measured from the tip of the nostril

to the root of the tail. BMI was calculated using the formula[61]

BMI = WEIGHT / LENGTH IN CM^2

ESTIMATION OF SERUM CHOLESTEROL AND TRIGLYCERIDES:

There was a linear correlation between the degree of obesity and plasma level of LDL cholesterol and triglycerides. Obesity and overweight are accompanied by unfavorable blood lipids patterns. Serum cholesterol and serum triglycerides were estimated using semi autoanalyser.[62]

ESTIMATION OF SERUM SOD:

Oxidantive stress (OS) has an essential role to play in the pathogenesis and progression of many diseases. OS results when the level of free-radical-formation is increased or protective antioxidant-mechanisms are compromised. Serum SOD was estimated with SOD determination kit purchased from sigma Aldrich. The principle used in this test was rate of reduction with oxygen was linearly related to xanthine Oxidase activity and was inhibited by SOD. The reagents used were WST working solution, enzyme solution and buffer solution and dilution buffer. WST working solution was prepared by diluting 1 ml of WST solution with 19 ml of buffer solution.20 µl of sample was added to each sample and and blank 2 well and double distilled water is added to blank 1 and blank 3 well. 20µL of enzyme working solution was added to each sample and blank1well and incubated at room temperature for 20 minutes. Absorbance was read at 450 nm using ELISA reader (BIORAD).[63]

ESTIMATION OF SERUM MDA

Obesity is a condition of chronic inflammation and oxidative stress. High fat diet will lead to oxidative stress reaction, thereby leading to obesity in rats.

Serum MDA was estimated with lipid per oxidation (MDA) assay kit purchased from sigma Aldrich (catalog number: MAK O85).

The reagents used were glacial acetic acid, Perchloric acid, Sulfuric acid and Thiobarbituric acid. MDA standard solution was prepared. 10 µL of sample was mixed with 500 µL of 42 Mm sulphuric acid and 125 µL of phosphotungstic acid. After mixing in vortex, incubated for 5 minutes at room temperature. Lipid per oxidation was determined by the reaction of MDA with Thiobarbituric acid to form a colorimetric product which was proportional to MDA.[64]

ESTIMATION OF SERUM LEPTIN, SERUM ADIPONECTIN, SERUM LIPASE, SERUM IL 6:

Serum leptin was estimated using double antibody sandwich enzyme linked immunosorbent assay technique. Elisa kits were purchased from Genosys laboratories, calalogue number: 201-11-0759/201-11-0456/201-11-0136/201-11-5135. The micro plate provided was pre-coated with specific antibody. Standards and samples when pipetted into the wells would bind to antibody that was immobile. After removal of the substance that was not bound, a biotin-conjugated antibody specific for serum leptin would be pipetted into the test wells. Subsequently Horseradish Peroxidase (HRP) was added and

incubated for 60 minutes at room temperature. An addition of substrate solution into the wells would lead on to color development that would be in proportion to bound serum leptin and then stop solution was added into each well to stop the reaction. Optic density was measured using ELISA reader(BIORAD) under 450 nm wavelength. Using similar method serum adiponectin, serum lipase and serum IL6 were estimated[65-72].

RESULTS

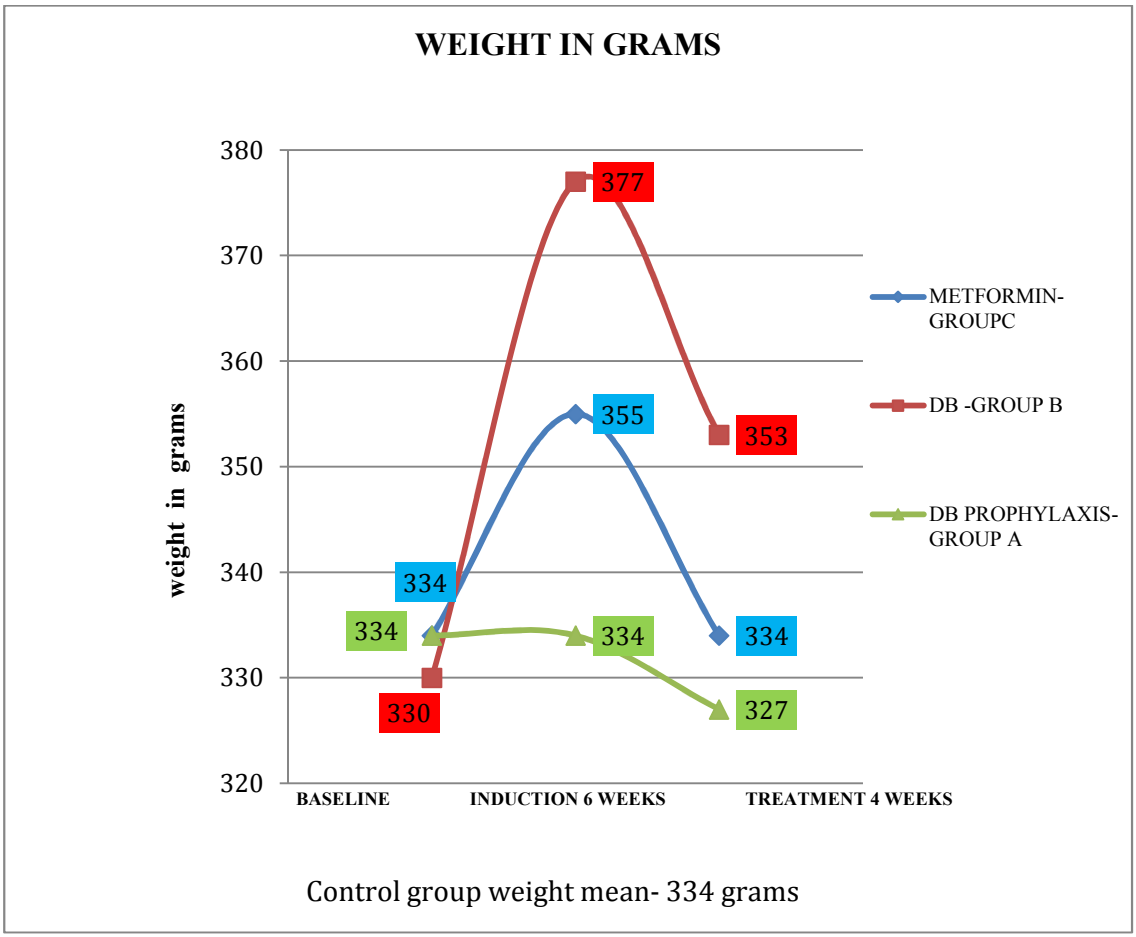
Using IBM-SPSS software version 24, results were analyzed using Paired T test and ANOVA post hoc test between the three groups

Table: 1 Mean values of control group

Serial Number	Parameters	Mean Values Of Control Group
1.	Food intake	14.66 grams
2.	Body Weight	334 grams
3.	BMI	1.045
4.	Serum SOD	0.195u/µl
5.	Serum MDA	0.318 nmol/l
6.	Serum leptin	7.244pg/ml
7.	Serum Adiponectin	7.705mg/l
8.	Serum IL6	11.53pg/ml
9.	Serum lipase	7.23ng/ml
10.	Serum cholesterol	522.8mg/dl
11.	Serum Triglycerides	203.01mg/dl

WEIGHT

Figure 3: Change In Weight Post Induction And Treatment



This picture shows that there was an increase in mean bodyweight of rats following induction and reduction in mean body weight after treatment in group B dolichos and group C Metformin group. In group A prophylaxis group there was no increase in mean bodyweight compared to baseline.(Table:1)

PAIRED T TEST:

Paired T test was assessed in all the three groups A, B, C baseline to induction and induction to treatment. Group A showed no increase in mean body weight after induction. Group B and Group C showed an increase in mean body weight after induction with high fat diet in comparison to baseline. Following treatment, Group A(prophylactic group) rats and Group B rats on cold extract of Dolichos showed a statistically significant reduction in body weight (p= 0.007,p=0.045) respectively. Group C rats on Metformin showed a reduction in mean body weight though not statistically significant. (Table: 2)

Table 2: Paired T test between baseline- induction & induction- treatment for Weight

Parameter	Groups	Pairs	Mean	SD	Significance
WEIGHT	A (prophylaxis)	Baseline induction	0.00000	7.53658	1.000
		Induction treatment	6.50000	3.67423	0.007
	B (Dolichos)	Baseline induction	47.00000	23.79916	0.005
		Induction treatment	24.16667	22.33756	0.045
	C (Metformin)	Baseline induction	20.66667	16.76504	0.029
		Induction treatment	21.33333	38.97521	0.238

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment in Group A, Group B and Group C in comparison to normal control values.

Following induction with high fat diet group B and C showed a significant increase in mean body weight compared to normal control. This indicates that obesity animal model was successfully induced. Group B was statistically significant ($p=0.002$). Following induction in prophylaxis Group A there was no significant increase in body weight in comparison to normal control. This indicates that prophylaxis with cold extract of dolichos prevented induction of obesity unlike the other 2 groups. Following treatment with cold extract of dolichos in group B and with Metformin in group C there was a reduction in mean body weight in both the groups B and C in comparison to the normal control. Though not statistically significant there was reduction in mean body weight. (Table: 1& 3)

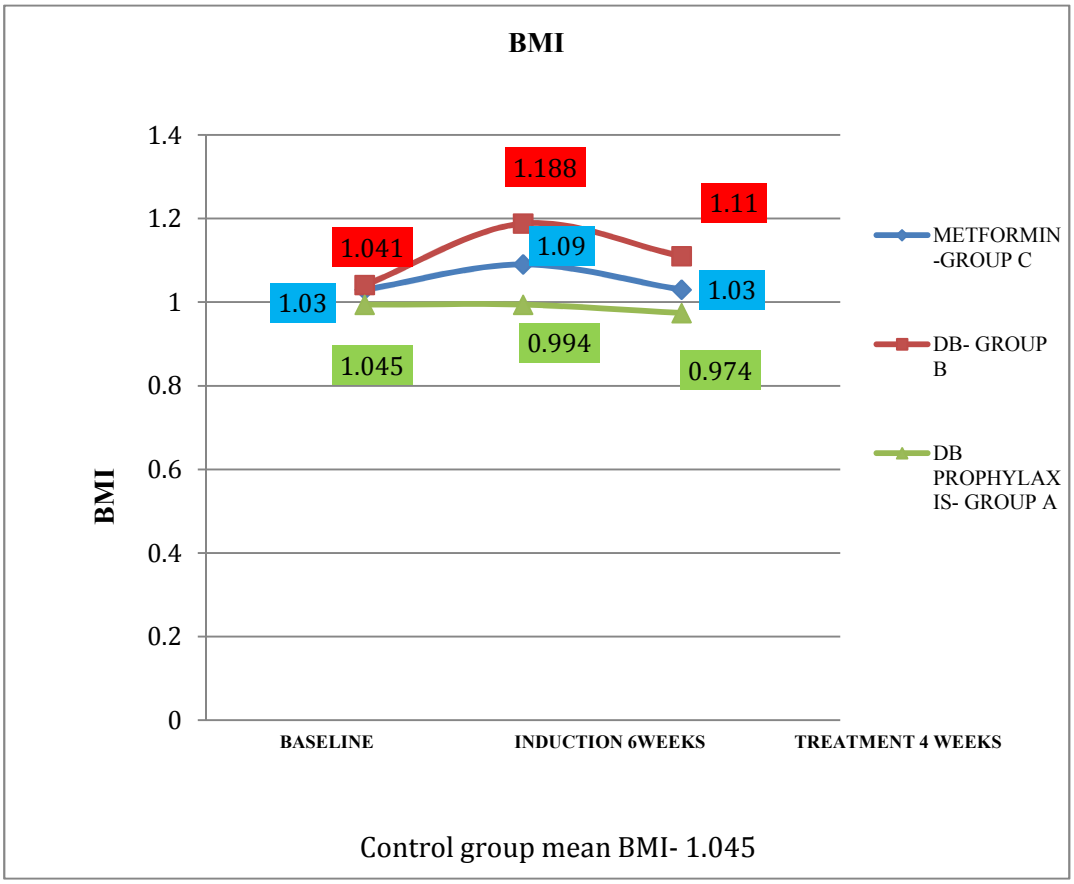
TABLE 3: ANOVA post hoc test between control and other groups for weight

Group	Group Induction	N	Mean	SD	P Value
Control	A(prophylaxis)	6	334.1667	28.18096	1.000
	B(Dolichos)	6	377.8333	24.81465	0.020

Group	Group Treatment	N	Mean	SD	P Value
Control	A(prophylaxis)	6	327.6667	30.50683	0.983
	B(Dolichos)	6	353.6667	29.45279	0.698
	C(Metformin)	6	334.1667	38.72166	1.000

BMI

Figure 4: Change In BMI Post Induction And Treatment



In this picture there was an increase in mean BMI of rats following induction and reduction in mean BMI after treatment in the dolichos group B and Metformin group C.

In the prophylaxis group A there was no increase in mean BMI compared to baseline.

PAIRED T TEST:

Paired T test was assessed in all the three groups group A, B, C baseline to induction and induction to treatment. Group A showed only mild increase in mean BMI after induction. Group B and Group C showed an increase in mean BMI after induction with high fat diet in comparison to baseline and it was significant in group B (P=0.004)

Following treatment, Group B rats on cold extract of dolichos showed a statistically significant reduction in BMI (p=0.041).Group A (prophylactic group) and Group C rats on Metformin showed a reduction in mean BMI though not significant.(Table:4)

TABLE 4: Paired T test between baseline-induction & induction-treatment for BMI

Parameter	Groups	Pairs	Mean	SD	Significance
BMI	A(prophylaxis)	Baseline induction	0.00050	0.02049	0.955
		Induction treatment	0.01917	0.01212	0.012
	B(Dolichos)	Baseline induction	0.14683	0.06993	0.004
		Induction treatment	0.07783	0.06954	0.041
	C(Metformin)	Baseline induction	0.05750	0.06104	0.069
		Induction treatment	0.05500	0.11286	1.194

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B,prophylaxis in Group A and with Metformin in Group C in comparison to normal control values**.**

Following induction with high fat diet group B and C showed a significant increase in mean BMI compared to normal control. This indicates that obesity animal model was successfully induced. Group B was statistically significant (p=0.001)

Following induction in prophylaxis Group A there was no significant increase in mean BMI in comparison to normal control. This indicates that prophylaxis with cold extract of dolichos prevented induction of obesity unlike the other 2 groups.

Following treatment with cold extract of dolichos in group B and with Metformin in group C there was a reduction in mean BMI in both the groups in comparison to the normal control BMI. Though not statistically significant there was reduction in mean BMI.

Following treatment with cold extract of dolichos in GROUP A prophylaxis group there was mean reduction in BMI in comparison to the normal control.(TABLE:1&5)

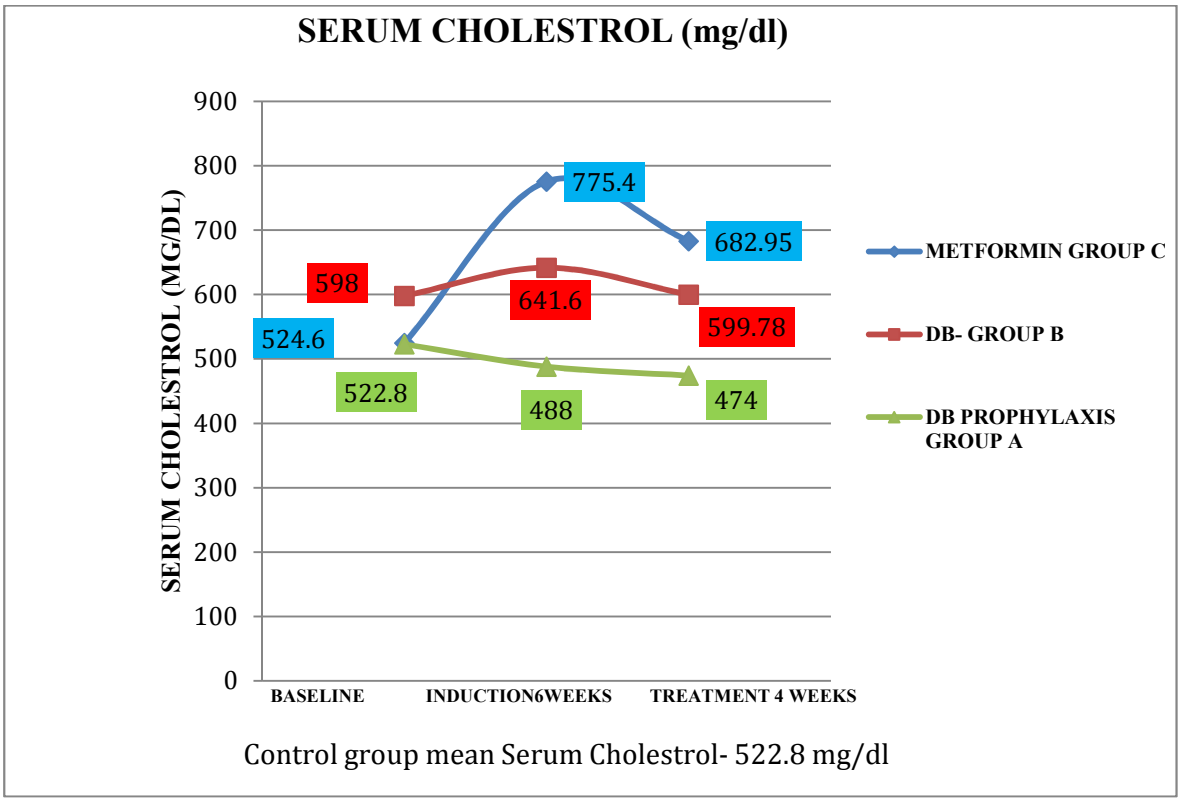
TABLE : 5 ANOVA post hoc test between control and other groups for BMI

Group	Group Induction	N	Mean	SD	P Value
Control	A(prophylaxis)	6	0.9935	0.08633	1.000
	B(Dolichos)	6	1.1880	0.08971	0.001
	C(Metformin)	6	1.0898	0.05382	0.157

Group	Group Treatment	N	Mean	SD	P Value
Control	A(prophylaxis)	6	0.9743	0.09154	0.987
	B(Dolichos)	6	1.1102	0.12777	0.240
	C(Metformin)	6	1.0348	0.11660	0.902

SERUM CHOLESTEROL (mg/dl)

Figure: 5 Serum Cholesterol Post induction &Treatment



This picture shows that there was an increase in mean serum cholesterol of rats following induction and reduction in mean serum cholesterol after treatment in the dolichos group B and Metformin C group.

In the prophylaxis group A there was no increase in mean serum cholesterol compared to baseline.

PAIRED T TEST:

Paired T test was assessed in all the three groups baseline to induction and induction to treatment.

Group A showed mild increase in mean total cholesterol after induction. Group B and Group C showed an increase in mean serum cholesterol after induction with high fat diet in comparison to baseline. Following treatment, Group B rats on cold extract, Group A prophylaxis and Group C rats on Metformin showed a reduction in mean serum cholesterol though not statistically significant.(Table:6)

TABLE: 6 Paired T test between baseline to induction and induction to treatment for serum cholesterol

Parameter	Groups	Pairs	Mean	Sd	Significance
Cholesterol	A(prophylaxis)	Baseline induction	34.88333	274.91401	0.768
		Induction treatment	13.76667	20.06416	0.154
	B(Dolichos)	Baseline induction	43.50000	140.96373	0.484
		Induction treatment	41.88167	130.65843	0.468
	C(Metformin)	Baseline induction	250.80000	194.68687	0.025
		Induction treatment	92.51667	136.98624	0.159

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B, prophylaxis in Group A and with Metformin in Group C in comparison to normal control values**.**

Following induction with high fat diet group B and C showed a significant increase in mean serum cholesterol compared to normal control. This indicates that obesity animal model was successfully induced. Group C was statistically significant (p=0.018)

Following induction in prophylaxis Group A there was no significant increase in serum cholesterol in comparison to normal control. This indicates that prophylaxis with cold extract of dolichos prevented induction of obesity unlike the other 2 groups.

Following treatment with cold extract of dolichos in group B and with Metformin in group C though there was a reduction in mean serum cholesterol it was not upto baseline value. Following treatment with cold extract of dolichos in GROUP A prophylaxis group there was reduction in mean serum cholesterol in comparison to the normal control. (Table:1&7)

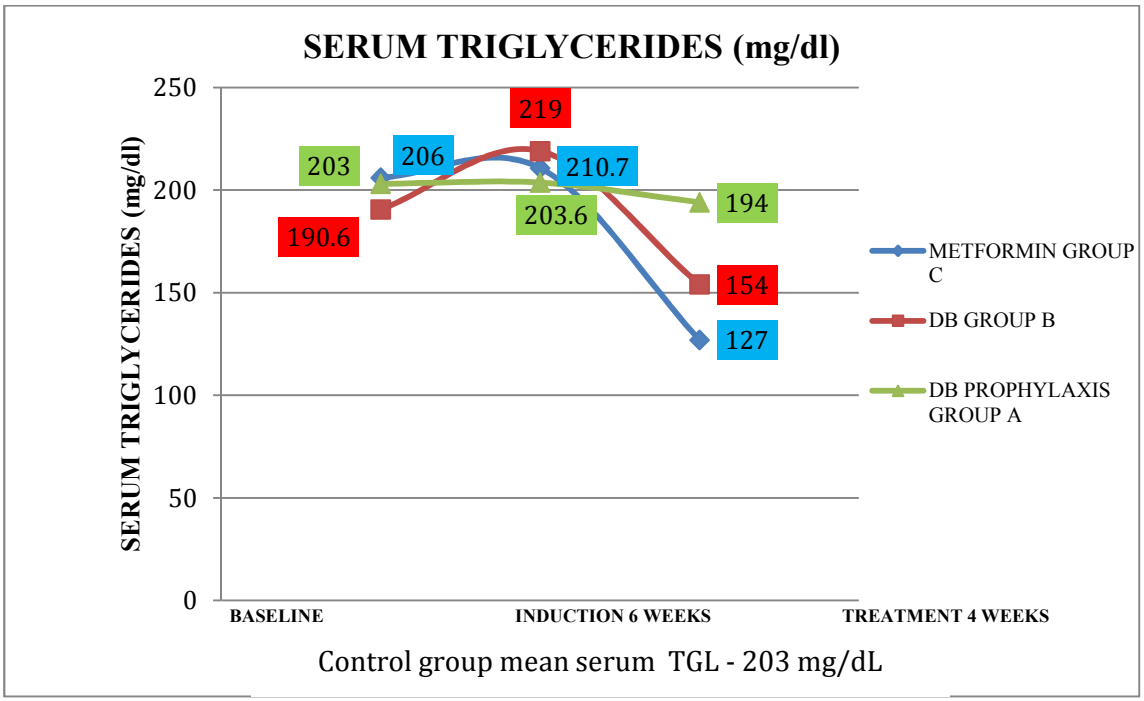
TABLE 7: ANOVA post hoc test between control and other groups for serum cholesterol

Group	Group Induction	N	Mean	SD	P Value
Control	A(prophylaxis)	6	487.9667	80.81241	0.968
	B(Dolichos)	6	641.6667	120.88200	0.432
	C(Metformin)	6	775.4667	83.41632	0.018

Group	Group Treatment	N	Mean	SD	P Value
Control	A(prophylaxis)	6	474.150	78.9387	0.932
	B(Dolichos)	6	599.7850	144.3347	0.783
	C(Metformin)	6	682.9500	082.9500	0.236

SERUM TRIGLYCERIDES (TGL) (mg/dl)

Figure 6: Change In Serum TGL Post Induction And Treatment



This picture shows that there was an increase in mean serum triglycerides of rats following induction and reduction in mean serum triglycerides after treatment in the dolichos group B and Metformin group C.In the prophylaxis group A increase in mean serum triglycerides was less compared to other groups.

PAIRED T TEST

Paired T test was assessed in all the three groups baseline to induction and induction to treatment. Group A showed no significant increase in mean serum triglycerides after induction. Group B and Group C showed an increase in mean serum triglycerides after induction with high fat diet in comparison to baseline. Following treatment, Group A rats and Group B rats on cold extract of dolichos, Group C rats on Metformin showed a reduction in mean serum triglycerides.(Table:8)

TABLE 8: Paired T test between baseline to induction and induction to treatment for serum triglycerides

Parameter	Groups	Pairs	Mean	SD	Significance
Triglycerides	A (prophylaxis)	Baseline induction	0.95000	274.91401	0.768
		Induction treatment	10.26667	20.06416	0.154
	B (Dolichos)	Baseline induction	28.51667	140.96373	0.484
		Induction treatment	65.16667	130.65843	0.468
	C (Metformin)	Baseline induction	4.48333	194.68687	0.025
		Induction treatment	83.66667	136.98624	0.159

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B, prophylaxis in Group A and with Metformin in Group C in comparison to normal control values.

Following induction with high fat diet group B and C showed an increase in mean serum triglycerides compared to normal control. This indicates that obesity animal model was successfully induced. Following induction in prophylaxis Group A there was no significant increase in serum triglycerides in comparison to normal control. This indicates that prophylaxis with cold extract of dolichos prevented induction of obesity unlike the other 2 groups.

Following treatment with cold extract of dolichos in group B and with Metformin in group C there was a reduction in mean serum triglycerides in both the groups in comparison to the normal control. Though not statistically significant there was reduction in mean serum triglycerides.

Following treatment with cold extract of dolichos in GROUP A prophylaxis group there was further reduction in mean serum triglycerides in comparison to the normal control.(table:1&9)

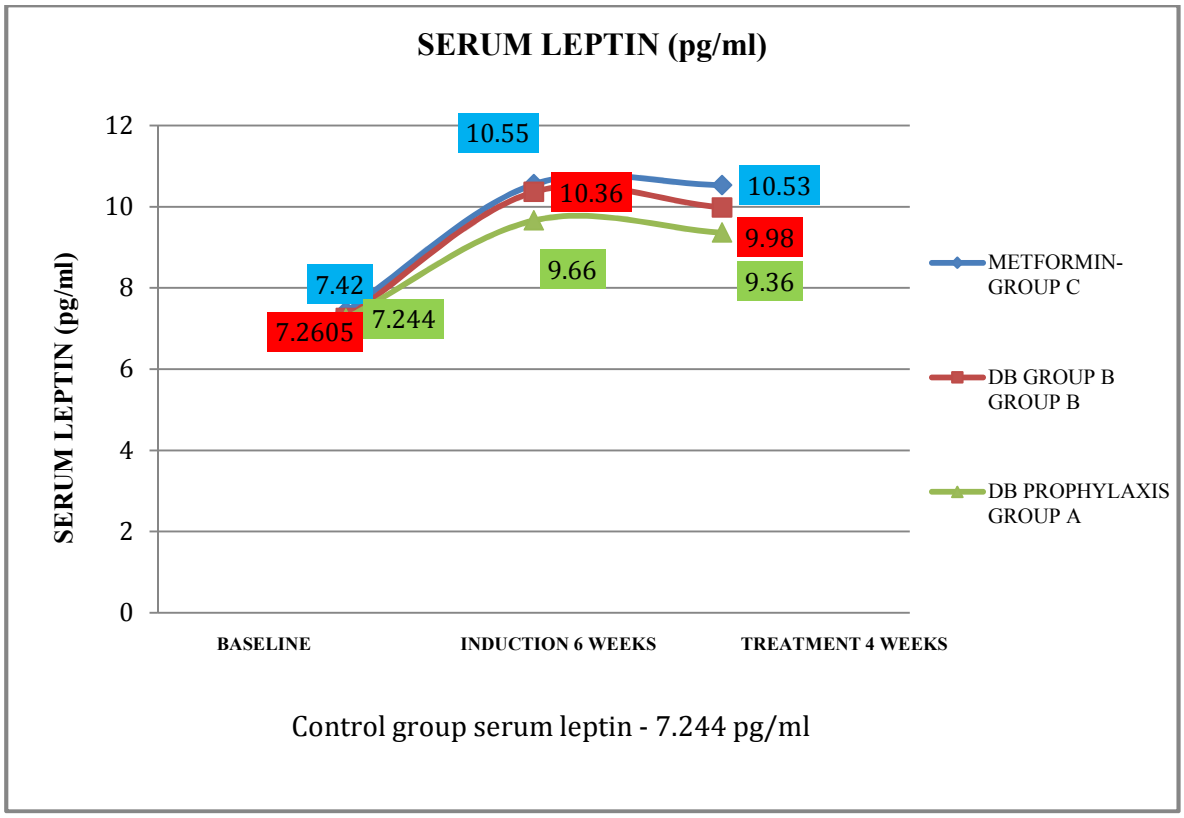
TABLE 9: ANOVA post hoc test between control and other groups for serum triglycerides

Group	Group Induction	N	Mean	SD	P Value
Control	A(prophylaxis)	6	203.9667	41.29597	1.000
	B(Dolichos)	6	219.1167	53.04215	0.972
	C(Metformin)	6	210.7167	74.08502	0.997

Group	Group Treatment	N	Mean	SD	P Value
Control	A(prophylaxis)	6	193.7000	41.29597	0.993
	B(Dolichos)	6	153.7000	53.04215	0.498
	C(Metformin)	6	127.0500	73.08502	0.155

SERUM LEPTIN (pg/ml)

FIGURE 7: Changes In Serum Leptin (Pg/Ml) Post Induction And Treatment



In this picture there was an increase in mean serum leptin of rats following induction and reduction in mean leptin after treatment in the dolichos group B and Metformin group C. In the prophylaxis group A there was increase in mean leptin but less than other groups compared to baseline.

PAIRED T TEST:

Paired T test was assessed in all the three groups baseline to induction and induction to treatment. Group A Group B and Group C showed an increase in mean serum leptin after induction with high fat diet in comparison to baseline though it was less in group A. This shows that obesity animal model was induced. Following treatment, Group A rats, Group B rats on cold extract of dolichos, and Group C rats on Metformin showed a reduction serum leptin(Table:10)

TABLE 10: Paired T test between baseline to induction and induction to treatment for serum leptin (pg/ml)

Parameter	Groups	Pairs	Mean	SD	Significance
LEPTIN	A(prophylaxis)	Baseline induction	2.41733	6.09649	0.376
		Induction treatment	0.29500	0.46011	0.177
	B(Dolichos)	Baseline induction	3.10083	6.37341	0.287
		Induction treatment	0.37417	3.99638	0.828
	C(Metformin)	Baseline induction	3.13033	4.73063	0.166
		Induction treatment	0.01817	2.55454	0.987

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with dolichos cold extract in Group B, prophylaxis in Group A and with Metformin in Group C in comparison to normal control values.

Following induction with high fat diet group B and C showed a significant increase in mean serum leptin compared to normal control. This indicates that obesity animal model was successfully induced. Following induction in prophylaxis Group A there was an increase in serum leptin in comparison to normal control but less than other two groups and reduction in mean serum leptin post induction. This indicates that prophylaxis with cold extract of dolichos was helpful in preventing induction of obesity unlike the other 2 groups.

Following treatment with cold extract of dolichos in group B and with Metformin in group C there was a reduction in mean serum leptin in both the groups B and C in comparison to the normal control. Though not statistically significant there was reduction in mean serum leptin. (Table: 1&11)

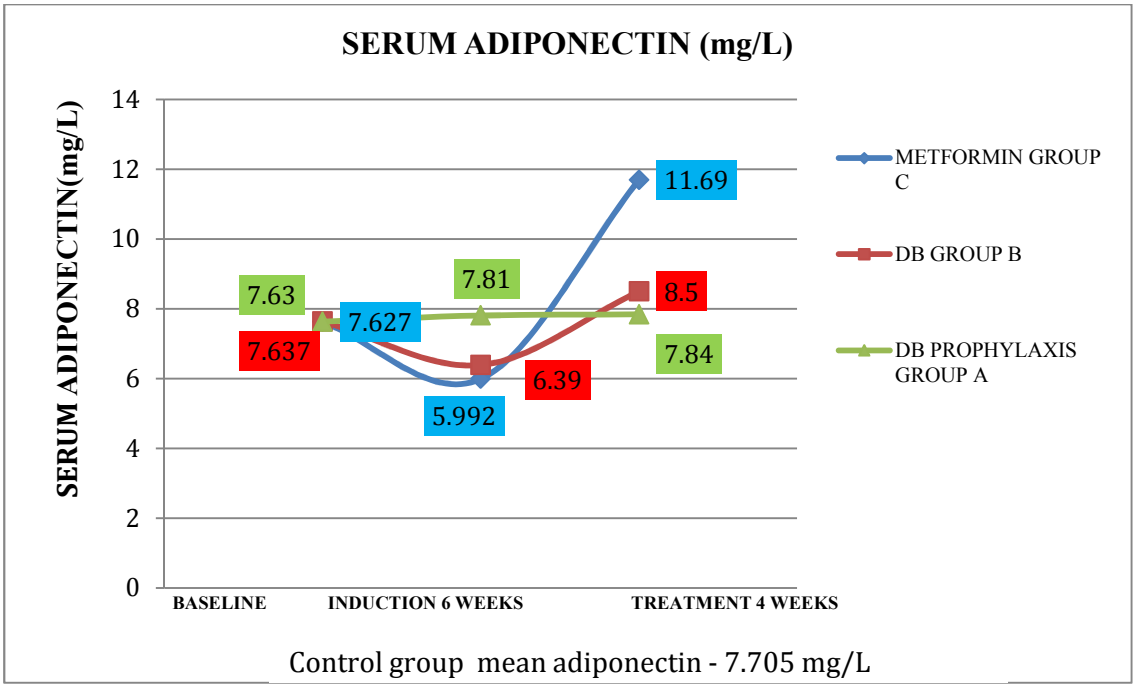
TABLE 11: ANOVA post hoc test for serum leptin between control and other groups

Group	Group Induction	N	Mean	SD	P Value
Control	A(prophylaxis)	6	9.6613	3.55783	0.484
	B(Dolichos)	6	10.3613	3.86984	0.679
	C(Metformin)	6	10.5515	3.02393	0.434

Group	Group Treatment	N	Mean	SD	P Value
Control	A(prophylaxis)	6	9.3663	3.69578	0.654
	B(Dolichos)	6	9.9872	1.81532	0.451
	C(Metformin)		10.5333	2.01639	0.298

SERUM ADIPONECTIN (mg/L)

Figure: 8 Changes In Serum Adiponectin (mg/L) Post Induction & Post Treatment



This picture shows that there was a decrease in mean serum adiponectin of rats following induction and increase in mean serum adiponectin after treatment in the dolichos group B and Metformin group C. In the prophylaxis group A there was an increase in mean serum adiponectin post induction and post treatment

PAIRED T TEST:

Paired T test was assessed in all the three groups baseline to induction and induction to treatment. Group A showed no significant reduction in mean serum adiponectin after induction. Group B and Group C showed a reduction in mean serum adiponectin after induction with high fat diet in comparison to baseline. Following treatment, Group A rats, Group B rats on cold extract of dolichos, Group C rats on Metformin showed an increase in mean serum adiponectin. Group C rats on Metformin showed a statistically significant increase in mean serum adiponectin (p=0.003) after treatment. (Table: 12)

TABLE 12: Paired T Test between baseline to induction & induction to treatment

Parameter	Groups	Pairs	Mean	SD	Significance
Adiponectin	A(prophylaxis)	Baseline induction	0.17900	1.20088	0.730
		Induction treatment	0.02483	0.03980	0.187
	B(Dolichos)	Baseline induction	1.24383	2.61118	0.296
		Induction treatment	2.11467	6.94792	0.489
	C(Metformin)	Baseline induction	1.63550	1.71464	2.336
		Induction treatment	5.69983	2.54476	0.003

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B,prophylaxis in Group A and with Metformin in Group C in comparison to normal control values.

Following induction with high fat diet group B and C showed a significant decrease in mean serum adiponectin compared to normal control. This indicates that obesity animal model was successfully induced. Following induction in prophylaxis Group A there was a increase in serum adiponectin in comparison to normal control .This indicates that prophylaxis with cold extract of dolichos prevented induction of obesity unlike the other 2 groups.

Following treatment with cold extract of dolichos in group B and with Metformin in group C there was an increase in mean serum adiponectin in both the groups B and C in comparison to the normal control. Though not statistically significant there was increase in mean serum adiponectin. (Table: 1 & Table: 12)

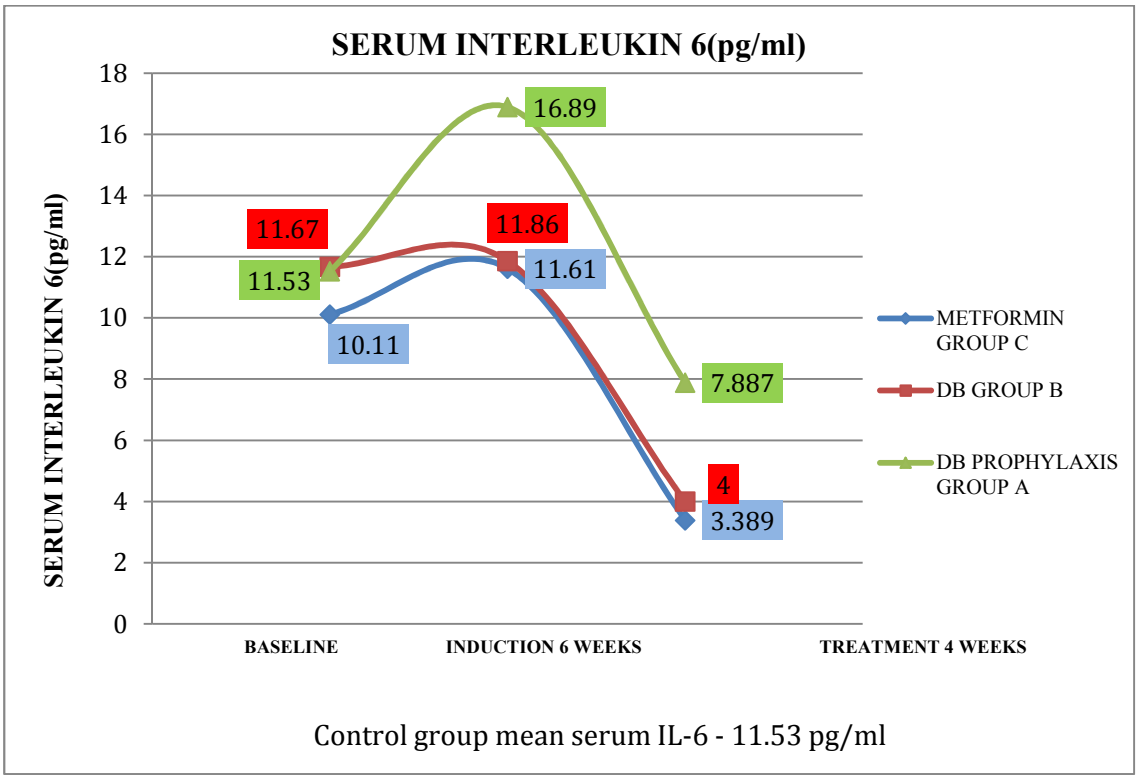
TABLE 13: ANOVA post hoc test between control and other groups for serum adiponectin

Group	Group Induction	N	Mean	SD	P Value
Control	A (prophylaxis)	6	7.8188	0.97846	0.995
	B (Dolichos)	6	6.3938	1.56669	0.362
	C (Metformin)	6	5.9923	0.51112	0.154

Group	Group Treatment	N	Mean	SD	P Value
Control	A (prophylaxis)	6	7.8437	1.0053	0.999
	B (Dolichos)	6	8.5085	5.08740	0.964
	C (Metformin)	6	11.6922	2.55395	0.157

SERUM INTERLEUKIN 6

Figure 9: Changes in Serum IL- 6 post induction & post treatment



In this picture there was an increase in serum IL6 following induction with high fat diet and decrease in serum IL6 following treatment with cold extract and Metformin in all the groups A, B and C

PAIRED T TEST:

Paired T test was assessed in all the three groups baseline to induction and induction to treatment. Prophylaxis Group A though showed an increase in mean serum IL6 post induction with high fat diet there was reduction in mean serum IL 6 in the treatment period .Group B and Group C showed an increase in mean serum IL6 after induction with high fat diet in comparison to baseline. Following treatment, Group A rats, Group B rats on cold extract of dolichos, Group C rats on Metformin showed a reduction in mean serum IL6.Group C rats on Metformin showed a maximum reduction in mean serum IL 6 after treatment. It was statistically significant for group B and C. (Table: 14)

TABLE 14: Paired Test between baseline to induction &induction to treatment for Serum IL-6

Parameter	Groups	Pairs	Mean	SD	P Value
IL 6	A(prophylaxis)	Baseline induction	5.36383	6.44275	0.097
		Induction treatment	9.00750	4.38387	0.004
	B(Dolichos)	Baseline induction	0.19067	0.96062	0.647
		Induction treatment	7.71117	3.12862	0.002
	C(Metformin)	Baseline induction	1.50200	1.63662	0.074
		Induction treatment	8.22450	2.05079	0.000

ANOVA:

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B, prophylaxis in Group A and treatment with Metformin in Group C in comparison to normal control values.

Following induction with high fat diet group B and C showed a significant increase in mean serum IL6 compared to normal control. This indicates that obesity animal model was successfully induced. Following induction in prophylaxis Group A though there was an increase in serum IL 6 post induction there was reduction in mean serum IL 6 in comparison to normal control.

Following treatment with cold extract of dolichos in group B and with Metformin in group C there was a reduction in mean serum IL6 in the groups B and C in comparison to the normal control. ($p=0.032$ and $p=0.016$) respectively. (Table: 1&15)

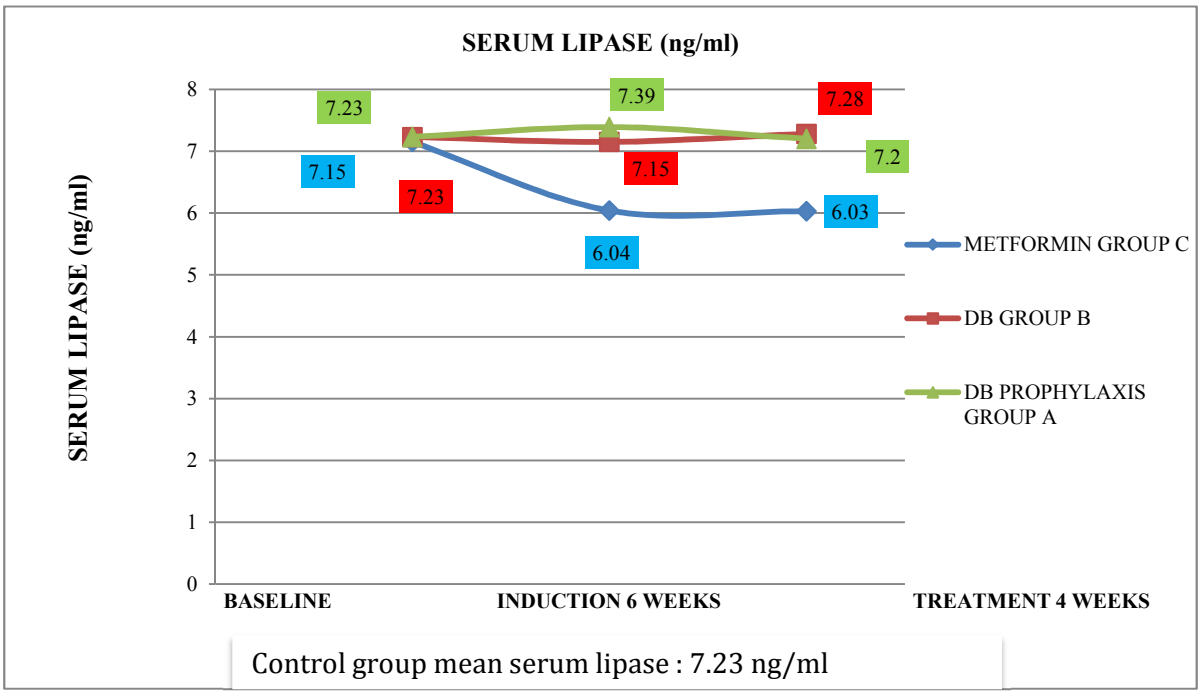
TABLE 15: ANOVA Post hoc test between control and other groups for serum IL6

Group	Group Induction	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	16.8952	3.38739	0.046
	B.(Dolichos)	6	11.8632	0.98317	0.998
	C.(Metformin)	6	11.6140	0.72161	1.000

Group	Group Treatment	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	7.8877	5.33883	0.462
	B.(Dolichos)	6	4.1520	2.75482	0.032
	C.(Metformin)	6	3.3985	2.40286	0.016

SERUM LIPASE (ng/ml)

FIGURE 10: Serum Lipase Post Induction & Post Treatment



In this picture there was a decrease in mean serum lipase after induction in group Band group C. In the prophylaxis group A there was a increase in mean serum lipase post induction and post treatment.

PAIRED T TEST:

Paired T test was assessed in all the three groups A, B, and C baseline to induction and induction to treatment. Group A showed an increase in mean serum lipase after induction with high fat diet. Group B and Group C showed a decrease in mean serum lipase after induction with high fat diet in comparison to baseline. Following treatment, Group A rats, Group B rats on cold extract of dolichos, Group C rats on Metformin showed an increase in mean serum lipase.(Table:16)

Table 16: **Paired T test baseline- induction &induction- treatment**

Parameter	Groups	Pairs	Mean	SD	Significance
LIPASE	A(prophylaxis)	Baseline induction	0.16100	1.49299	0.802
		Induction treatment	0.19017	0.39788	0.294
	B(Dolichos)	Baseline induction	0.07850	3.54128	0.959
		Induction treatment	1.3167	3.96603	0.938
	C(Metformin)	Baseline induction	1.11500	1.29959	0.090
		Induction treatment	0.00983	1.43941	0.987

ANOVA :

Analysis of variance was assessed in all the three groups following induction with high fat diet and treatment with cold extract in Group B, prophylaxis in Group A and treatment with Metformin in Group C in comparison to normal control values**.**

Following induction with high fat diet group B and C showed a significant decrease in mean serum lipase compared to normal control. This indicates that obesity animal model was successfully induced. Following induction with prophy laxis in Group A there was an increase in mean serum lipase in comparison to normal control.

Following treatment with cold extract of Dolichos in group B and with with Metformin in group C though there was an increase in mean serum lipase it was not upto baseline value.

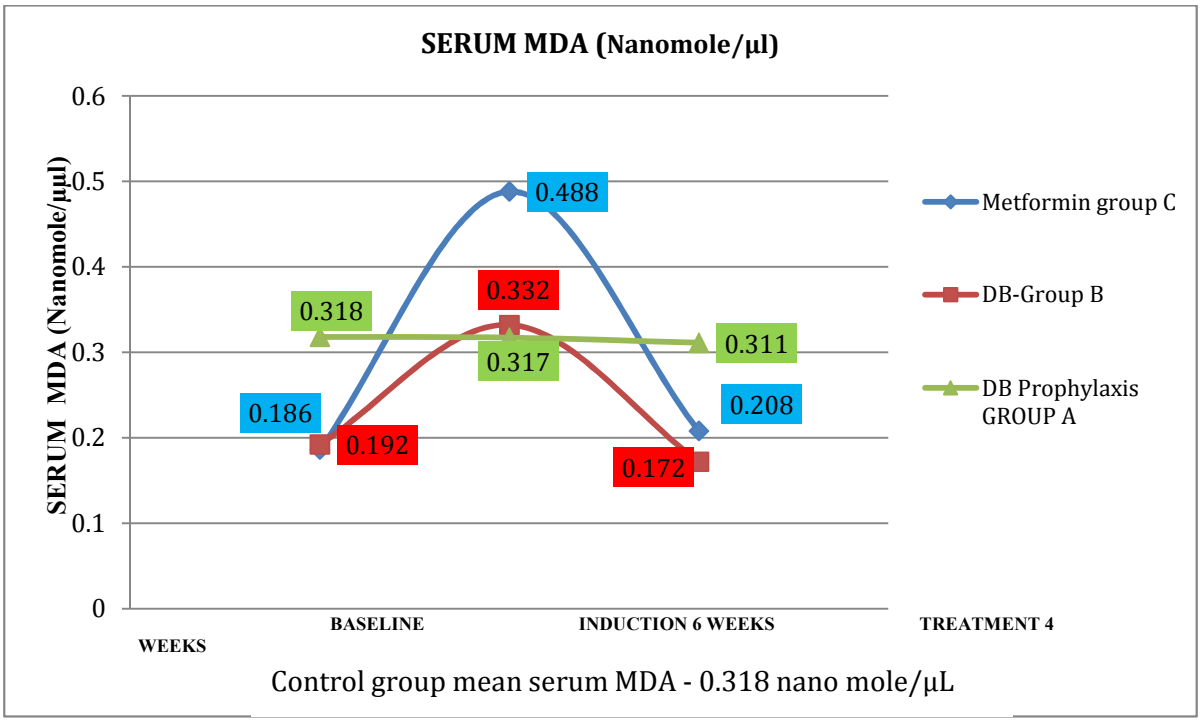
TABLE17: ANOVA Post hoc test for serum lipase between control & other groups

Group	Group Induction	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	7.3922	0.88252	0.999
	B.(Dolichos)	6	7.1482	3.12059	1.000
	C.(Metformin)	6	6.0392	1.03710	0.051

Group	Group Treatment	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	7.2020	0.92113	1.000
	B.(Dolichos)	6	7.2798	2.72752	1.000
	C.(Metformin	6	6.0293	0.80420	0.554

SERUM MDA (nanomol/µl)

Figure: 11 Serum MDA post induction &post treatment



In this picture there was an increase in mean serum MDA following induction in group B dolichos and group C Metformin group and decrease in mean serum MDA following treatment. In prophylaxis group A change from baseline is minimal.

PAIRED T TEST: SERUM MDA

Paired T test was assessed in all the three groups A, B and C baseline to induction and induction to treatment. Group A showed a decrease in mean serum MDA post induction with high fat diet. Group B and Group C showed an increase in mean serum MDA after induction with high fat diet in comparison to baseline. Following treatment, Group A rats, Group B rats on cold extract of Dolichos, Group C rats on Metformin showed a decrease in mean serum MDA. It was statistically significant for group B (P=0.001). (Table:1&18)

TABLE 18: Paired T test baseline- induction &induction- treatment for serum MDA

Parameter	Groups	Pairs	Mean	SD	Significance
MDA	A(prophylaxis)	Baseline induction	0.00100	0.25385	0.993
		Induction treatment	0.00583	0.00500	0.035
	B(Dolichos)	Baseline induction	0.14100	0.06157	0.002
		Induction treatment	0.16017	0.05584	0.001
	C(Metformin)	Baseline Induction	0.30167	0.77165	0.382
		Induction treatment	0.32583	0.76578	0.345

ANOVA MDA

Analysis of variance was assessed in all the three groups Group A, Group B and Group C following induction with high fat diet and treatment with cold extract in group B prophylaxis in group A and Metformin in group C in comparison to normal control.

Following induction with high fat diet group B and C showed a significant increase in mean serum MDA compared to normal control. This indicates that obesity animal model was successfully induced. Following induction with prophylaxis in Group A there was a decrease in mean serum MDA in comparison to normal control.

Following treatment with cold extract of dolichos in group A and group B and with Metformin in group C there was a decrease in mean serum MDA in the groups A,B and C in comparison to normal control though not statistically significant.(Table:1&19)

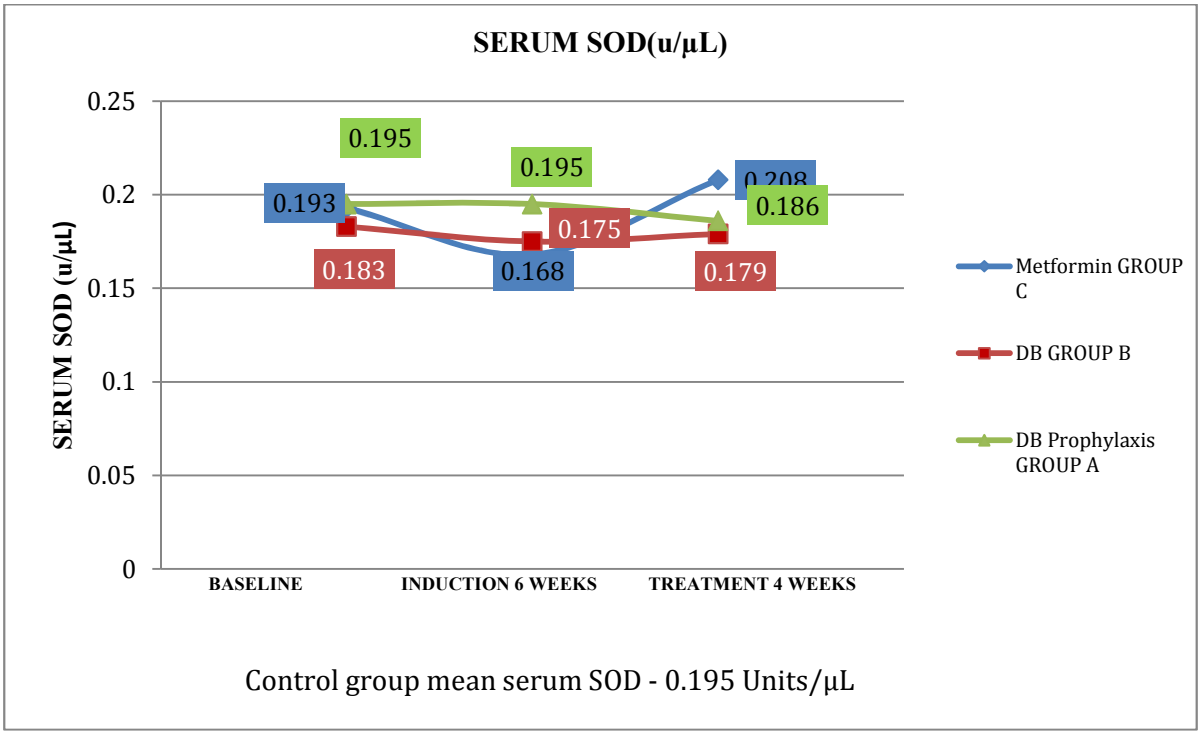
TABLE 19: ANOVA post hoc test for serum MDA between control & other groups

Group	Group Induction	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	0.3172	0.05471	1.000
	B.(Dolichos)	6	0.3330	0.02091	1.000
	C.(Metformin)	6	0.4880	0.75701	0.884

Group	Group Treatment	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	0.3113	0.05669	1.000
	B.(Dolichos)	6	0.1728	0.05724	0.341
	C.(Metformin)	6	0.1622	0.06879	0.283

SERUM SOD (u/µL)

FIGURE 12: SERUM SOD POSTINDUCTION & POSTTREATMENT



In this picture mean serum SOD was reduced after induction with high fat diet and increased after treatment with cold extract of Dolichos and with Metformin in group B&C.

PAIRED T TEST:

Paired T test was assessed in all the three groups A, B and C baseline to induction and induction to treatment. Group A showed a decrease in mean serum SOD after induction with HFD. Group B and Group C showed a decrease in mean serum SOD after induction with HFD in comparison to baseline. Following treatment, Group B rats on cold extract of dolichos, Group C rats on Metformin showed an increase in mean serum SOD. It was statistically significant for group C (p =0.017) (Table:20)

TABLE20: Paired T test baseline- induction &induction- treatment for serum SOD

Parameter	Groups	Pairs	Mean	SD	Significance
SOD	A(prophylaxis)	Baseline induction	0.00040	0.02259	0.967
		Induction treatment	0.00693	0.00516	0.022
	B(Dolichos)	Baseline induction	0.00662	0.09878	0.876
		Induction treatment	0.00435	0.05795	0.861
	C(Metformin)	Baseline induction	0.02532	0.3336	0.122
		Induction treatment	0.04023	0.02816	0.017

ANOVA

Analysis of variance was assessed in all the three groups Group A, Group B and Group C following induction with high fat diet and treatment with cold extract in group B,prophylaxis in group A and with Metformin in group C in comparison to normal control.

Following induction with high fat diet group B and C showed a significant decrease in mean serum SOD compared to normal control. This indicates that obesity animal model was successfully induced. Group A showed no decrease in mean serum SOD during induction and there was increase in mean serum SOD post induction.

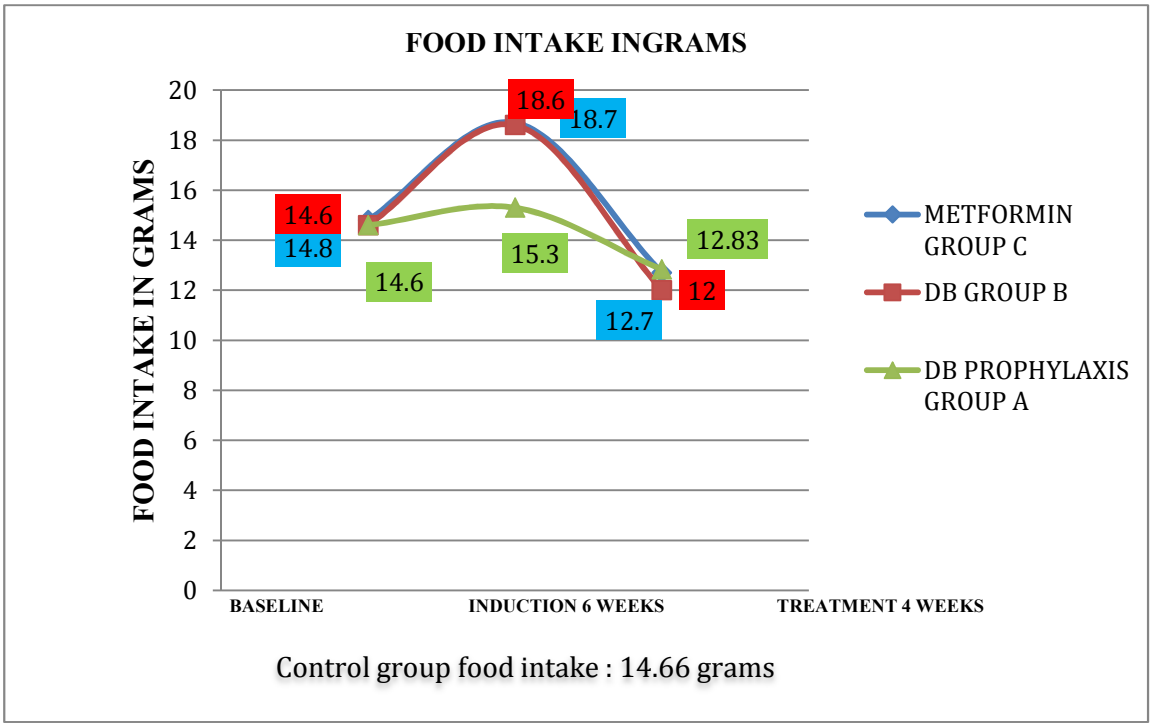
Following treatment with cold extract of dolichos in group B and with Metformin in group C and though there was a increase in mean serum SOD it was not upto baseline in value. (Table:1&21).

TABLE 21: ANOVA post hoc test for serum SOD between control & other groups

Group	Group Induction	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	0.1948	0.0178	1.000
	B.(Dolichos)	6	0.1756	0.04247	0.609
	C.(Metformin)	6	0.1684	0.03502	0.424

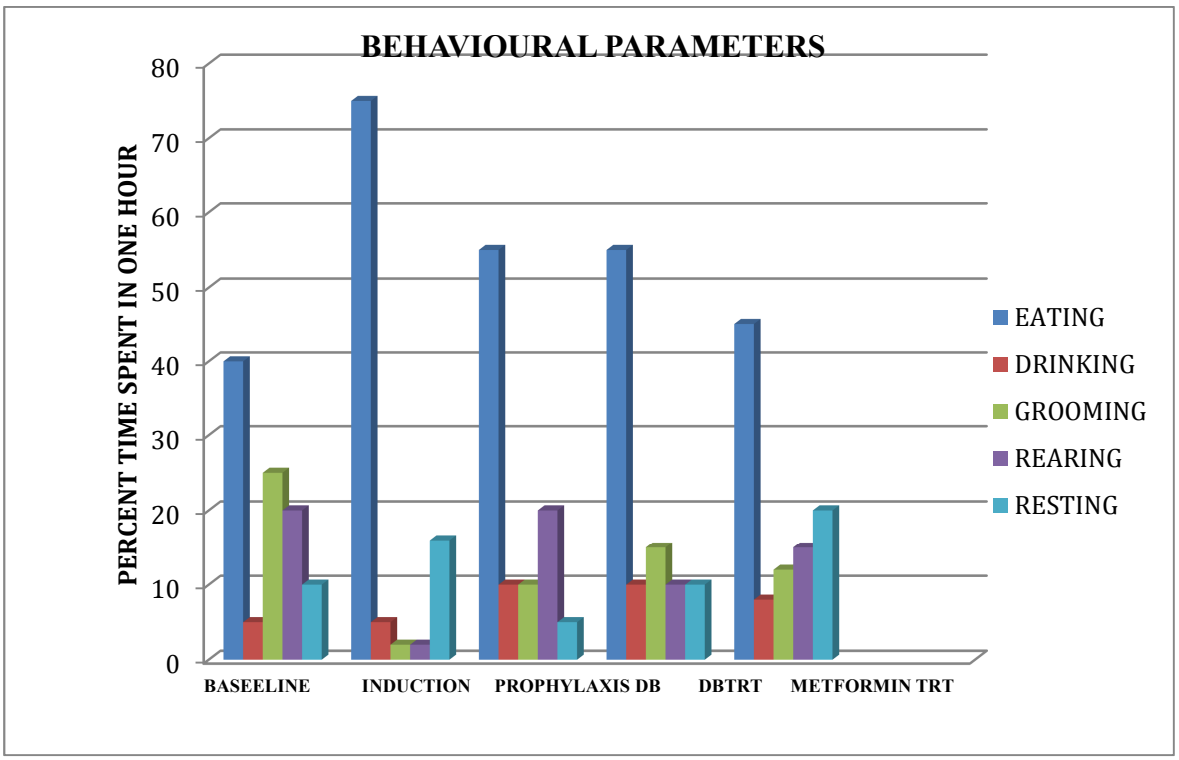
Group	Group Treatment	N	Mean	SD	P Value
CONTROL	A(prophylaxis)	6	0.1879	0.01551	0.978
	B.(Dolichos)	6	0.1799	0.05921	0.840
	C.(Metformin)	6	0.2087	0.01036	0.883

FIGURE 13: FOOD INTAKE (grams)



In this picture food intake was increased followed induction with high fat diet in all groups and reduced after treatment with Dolichos Biflorus and Metformin. Food intake was comparatively less in the prophylaxis group.

FIGURE 14: Behavioral changes



Animals were observed for activities like eating, drinking, grooming, rearing and resting at baseline, during induction and after treatment.

Percent of time spent in minutes for each behavior were compared to baseline during induction of obesity and following treatment with Dolichos and Metformin.

Percent of time spent for eating was high compared to other groups during obesity induction.

Percent of time spent in grooming , rearing, resting, drinking were less compared to baseline during induction and it again increased following treatment with dolichos and Metformin which explain some involvement with CNS function(Fig 14) .

DISCUSSION

Obesity is defined as abnormal or excessive fat accumulation that may impair health. WHO defines overweight as BMI equal to or more than 25 and obesity as BMI equal to or more than 30[13].In India, obesity is emerging as an important health problem particularly in urban areas. In a community based cross-sectional study prevalence of generalized, abdominal and combined obesity in India was found to be 56%, 71.2% and 51.3% respectively[73]. The Chennai urban rural epidemiology study (CURES) conducted in Chennai city in Tamil Nadu reported age standardized prevalence of generalized obesity to be 45.9 per cent, while that of abdominal obesity was 46.6 percent.[74]

Our hypothesis was that cold extract of Dolichos biflorus has weight reducing property and the weight reducing property may be due to central mechanism, anti-inflammatory effects, antioxidant property or metabolic effects.

According to the study protocol 18 male Swiss albino rats were selected and were divided into three groups of 6 animals each. After observing the baseline parameters obesity was induced by high fat diet for a period of 6 weeks. Group A was the prophylactic group in which cold extract was given along with high fat diet for a period of 6 weeks and followed for a period of another 4 weeks. Group B was the Dolichos group in which obesity was induced by high fat diet for a period of 6 weeks followed by treatment with cold extract of Dolichos for 4 weeks. Group C was the Metformin group in which obesity was induced by high fat diet for 6 weeks followed by treatment

with Metformin 2 mg per 100 gram per day for a period of 4 weeks. Post induction and post treatment parameters were taken. Blood samples were taken for analysis.

In a previous study Piper betel leaf extract and Dolichos biflorus seed extract showed potent anti-adipogenic efficacy. Combination of Piper betel leaf extract and Dolicho*s* biflorus seed extract in a ratio of 2:3, LI10903F also known as LOWAT demonstrated greater anti-adipogenic and lipolytic activities compared to the individual extracts[40]. Hypolipidemic effect of Dolichos was also seen in this study. Serum cholesterol and serum triglycerides increased after induction with high fat diet and decreased after treatment with cold extract and was equally effective as Metformin (Table-5, 6,7,8) & (Figure-5 &6).

In a clinical study hot extract of Dolichos was given to overweight or obese male and female human volunteers. Pre and post treatment bodyweight was assessed. It was found that hot extract of Dolichos has better antiobesity activity. The onset of antiobesity effect was quicker in males than females[41]. In this study we found that after induction with high fat diet there was a marked increase in mean body weight in both the Dolichos group B and Metformin group C and there was a reduction in mean body weight after treatment with Dolichos cold extract and Metformin. There was also a corresponding increase in mean BMI during induction and reduction in mean BMI after treatment in both the groups B & C In the prophylactic group A there was no significant increase in mean body weight and BMI. (Figure-3 & 4) & (Table- 1, 2,3 & 4)

In an animal study with adult albino rats of Wistar strain it was proved that cold extract of Dolichos biflorus seeds possesses anorectic property[42]. In this study food intake by rats was measured at baseline, post induction with high fat diet and post treatment with cold extract of Dolichos and Metformin. Food intake was reduced in both the groups A & B after treatment compared to the induction period.(fig13& fig14). This shows the anorectic effect of Dolichos.

In rodents, grooming is a complex phenomenon which normally proceeds in a cephalocaudal direction and consists of several stages including licking the paws, washing movements over the head, fur licking and tail/genitals cleaning. Grooming is highly sensitive to various stressors, psychotropic drugs and genetic manipulations[75]. We observed the animals for 1 hour for activities like eating, drinking, grooming, rearing and resting before induction, during induction and after treatment. The time spent for eating in percentage was more when compared to other activities during the period of induction. There was reduction in time spent for eating after treatment in both the groups B & C (Figure- 14)

Other parameters like serum SOD, serum MDA, serum Leptin, serum Adiponectin, serum cholesterol, serum triglycerides, serum IL6 and Serum Lipase were analysed. Reactive oxygen species (ROS) and oxidative stress in adipose tissue play a major role in the development of obesity. Superoxide dismutase (SOD) is an enzyme responsible for the maintenance of oxidation reduction homeostasis which protects against high fat diet induced obesity and its complications[76] In this study there was a decrease in serum SOD in rats

following induction with high fat diet in the Metformin group C and Dolichos group B and SOD level increased following treatment with cold extract of Dolichos and Metformin in both the treatment groups B & C. In the prophylactic group A there was no difference during induction and post induction. (Figure:12 Table:19 & Table:20)

Obesity is associated with an increased risk of developing atherosclerosis. Oxidative modification of lipoproteins may play an important role in the pathogenesis of atherosclerosis. Malondialdehyde (MDA) is one of the indicators of lipid per oxidation. Obesity in humans is an independent risk factor for lipid per oxidation and depletion of cytoprotective enzymes even in the absence of other confounding factors such as diabetes and hyperlipidaemia. Over a period of time antioxidant enzymes are depleted and cannot cope with increasing oxidative stress[77]. In this study there was an increase in mean serum MDA in rats following induction with high fat diet and mean serum MDA level decreased following treatment with cold extract of Dolichos and Metformin in group B & C. In the prophylactic group A there was reduction in MDA during induction and further reduction in MDA post induction (Table- 17 & 18) & (Figure-11)

Adiponectin is an adipokine secreted by adipose tissues. It has antidiabetic, anti-inflammatory antiatherogenic and cardio protective effects. Adiponectin expression and serum levels are reduced in obese patients and increased after weight loss[78]. In this study there was reduction in serum adiponectin in rats following induction with high fat diet and adiponectin level

increased following treatment with cold extract of dolichos and Metformin in group B &C. In the prophylactic group A there was an increase in serum Adiponectin during induction. (Table-11 & 12) & (Figure-8)

Lipase is a central enzyme in lipid metabolism which is synthesized and secreted by adipocytes and muscle. In obesity lipid accumulates and stored in ectopic sites such as liver and muscle resulting in insulin resistance[79]. In this study there was reduction in mean serum lipase in rats following induction with high fat diet and mean serum lipase level increased following treatment with cold extract of Dolichos and Metformin. In the prophylactic group A similar effect was seen. (Table:15& 16 Figure : 10)

Interleukin-6 is the principal procoagulant cytokine. It increases plasma concentrations of fibrinogen, plasminogen activator inhibitor type 1 and C reactive protein. An elevated level of IL-6 is associated with increased risk of myocardial infarction in healthy men[80]. In this study there was an increase in mean serum IL6 following induction the groups B and C and reduction in mean serum IL6 following treatment with cold extract of Dolichos and Metformin. In prophylaxis group A though there was increase in mean serum IL 6 in the induction period there was reduction in mean serum IL 6 after treatment. This shows the anti-inflammatory effects of cold extract of Dolichos and Metformin. (Table: 13 & 14 Figure: 9)

Leptin is an adipokine involved in satiety regulation and obesity. Leptin is primarily expressed in adipose tissue and studies in mice show a central role for leptin in food intake and regulation of energy balance. Leptin-deficient

mice (ob=ob mice) or leptin-receptor deficient mice (db=db mice) are more prone to develop obesity. Administration of leptin to leptin deficient mice increases energy expenditure, decreases body weight and normalizes hyperglycemia, insulin resistance and hyperinsulinemia. Human obesity is characterized by increased plasma leptin concentrations. [81] In this study there was an increase in mean serum leptin in rats following induction with high fat diet and reduction in mean serum leptin level following treatment with cold extract of dolichos and Metformin in groups B and C.(Table:9&10 Figure :7).

To conclude based on analysis of this study weight and BMI of rats increased following induction with high fat diet. There was also decrease in mean serum SOD and increase in mean serum MDA following induction. This shows the oxidative stress associated with obesity. There was also increase in mean serum leptin, decrease in mean serum adiponectin, decrease in mean serum lipase, increase in mean serum IL 6 following induction with high fat diet and reversal of effects following treatment with cold extract of Dolichos biflorus and Metformin.

CONCLUSIONS

In this animal study effects of cold extract of Dolichos (12 hours extract) in reducing high fat diet induced obesity in rats was analyzed and was compared with that of Metformin.

Cold extract of Dolichos has anorectic property as evidenced by reduced food intake. Food intake of rats increased following induction with high fat diet and it was reduced after treatment with cold extract of Dolichos.

This study proved the anti-inflammatory property of cold extract of Dolichos. The inflammatory marker serum Interleukin6 was increased after induction with high fat diet and it was reduced after treatment with cold extract of Dolichos. Obesity is a condition of oxidative stress and cellular inflammation. Thus cold extract of Dolichos can be used as an adjuvant agent to treat this condition.

Cold extract of Dolichos has antioxidant property. SOD and MDA are biomarkers related to oxidative stress. Following induction with high fat diet mean serum SOD level decreased and mean serum MDA level increased. After treatment with cold extract of Dolichos mean serum SOD level increased and mean serum MDA level decreased thus proving the antioxidant property of cold extract of Dolichos.

Weight and BMI of rats were analyzed at baseline post induction and post treatment with cold extract of Dolichos. There was an increase in mean bodyweight and BMI following induction with high fat diet and reduction in mean body weight and BMI following treatment with cold extract of Dolichos.

Cold extract of Dolichos has hypolipidemic effect. Mean serum cholesterol and mean serum triglycerides increased following induction with high fat diet and it was reduced after treatment with cold extract of Dolichos.

In this study mean serum leptin increased following induction with high fat diet and decreased following treatment with cold extract of Dolichos. Obesity is a condition of leptin resistance and this study proved the central mechanism of antiobesity effect of Dolichos. Adiponectin is antiatherogenic cytokine and has cardio protective effects. In this study there was a decrease in serum adiponectin after induction with high fat diet and there was an increase in mean serum adiponection after treatment with cold extract of Dolichos .This shows the cardio protective effects of Dolichos.

Lipase which is a key enzyme in lipid metabolism decreased after induction with high fat diet and increased after treatment with cold extract of Dolichos thus proving that antiobesity effect of Dolichos may be due to peripheral mechanism of action.

Thus we conclude that cold extract of Dolichos has antiobesity effect due to central and peripheral mechanism of action. It also has anti-inflammatory, antioxidant and hypolipidemic effect. This study explained clearly about the antiobesity effects of 12 hours cold extract of Dolichos and it was equally effective and comparable to that of Metformin. Thus cold extract of Dolichos can be used as a household remedy for the treatment of obesity as it is cheap and easily available and without any adverse effects. With the suitable clinical study it may be useful in reducing bodyweight in humans in future.

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ANNEXURE

ABBREVATIONS

AgRP	-	Agouti related peptide
BMI	-	Body mass index
CART	-	Cocaine amphetamine related transcript
CB I receptor	-	Cannabinoid receptor type I
CCK	-	Cholecystokinin
CHD	-	Coronary heart disease
COX	-	Cyclooxygenase
CRH	-	Corticotrophin releasing hormone
DME	-	Methanolic extract of dolichos
FFA	-	Free fatty acids
GLP 1	-	Glucagon like polypeptide
GnRH	-	Gonadotropin releasing hormone
HDL	-	High density lipoprotein
HFD	-	High fat diet
HPG	-	Hypothalamic pituitary gonadal axis
IARC	-	International agency for research on cancer
IGF BP	-	Insulin like growth factor binding protein
IGF-1	–	Insulin like growth factor
IL6	-	Interleukin 6
LDL	-	Low density lipo protein
MC3R	-	Melanocortin 3 receptor

MDA	-	Malondialdehyde
MSH	-	Melanin stimulating hormone
NOS	–	Nitric oxide synthase
NPY	-	Neuropeptide Y
NZO mouse	-	New Zealand obese mouse
OLEFT rat	-	Otsuka-Long-Evans-Tokushima-Fatty rat
OS	-	Oxidative stress
OSA	_	Obtructive sleep apnea
PCOS	-	Polycystic ovarian syndrome
POMC	–	Proopiomelanocortin
PYY3- 36	-	Polypeptide YY- 3-36
SNS	-	Sympathetic nervous system
SOD	-	Super oxide bismutasce
SSRI	-	Selective serotonin reuptake inhibbitor
TNF α	-	Tumour necrosis factor
VLDL	-	Very low density lipoprotein
WST	-	Water soluble tetrazolium salt

EATING BEHAVIOUR

TIME IN MINUTES	FEEDING	GROOMING	REARING	DRINKING	RESTING
observations are made for a period of one hour					

Group	Baseline weight	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11