PHARMACOLOGICAL EVALUATION OF DOPAMINE CONTAINING PLANTS IN OBESITY

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

by

Javid I. Mansuri

Enrollment No.: 119997290022

under supervision of

Dr. Archana N. Paranjape

GUJARAT TECHNOLOGICAL UNIVERSITY
AHMEDABAD

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ABSTRACT

Pharmacological Evaluation of Dopamine containing plants in obesity

Objective

Dietary imbalance and over nutrition may lead to diseases like obesity. Obesity results when caloric intake exceeds utilisation. Dopamine precursor L-DOPA and dopamine D2 receptor agonists are reported to have weight reducing property. They have also shown significant decrease in food intake in normal as well as high fat diet fed rats. The present study was aimed at evaluating the antiobesity potential of three dopamine containing plants in high fat diet induced obesity in rats.

Methods

Male Sprague Dawley (SD) rats were divided in five groups of 6 animals in each for each test drug. Group 1 served as control and was given normal pellet diet while all other groups were subjected to high fat diet (HFD) for 12weeks. L-DOPA (12.5 mg/kg, p. o.) as standard drug and aqueous extract of *Mucuna pruriens* seeds (AEMP 200 mg/kg, p. o. and 400 mg/kg, p. o.), aqueous extract of *Vicia faba* seeds (AEVF 300 mg/kg, p. o. and 600 mg/kg, p. o.), and aqueous extract of *Bauhinia purpurea* seeds (AEBP 300 mg/kg, p. o. and 600 mg/kg, p. o.) as test drugs were administered in separate groups in last 4 weeks along with HFD. Body weight, food intake, body mass index (BMI), serum total cholesterol(TC), triglyceride (TG)and high density lipoprotein (HDL) levels were measured at the end of fourth , eighth and twelfth weeks, while white adipose tissue (WAT) mass and brain dopamine levels were measured at the end of the twelfth weeks.

Results

Body weight reduction is the key parameter to check anti-obesity potential of any drug. In present study, HFD-induced animals showed a mean body weight gain of 46.67 g ($p<0.01$) as compared to normal diet administered animals after 12 weeks of induction period. L-DOPA and AEMP (400 mg/kg) treated group showed a mean loss of 23.00 g ($p<0.001$) and 30.00 g ($p<0.001$) body weight respectively. In AEMP (200 mg/kg) treated groups mean body weight gain was found to be 6.66 g ($p<0.01$). AEVF (600 mg/kg) treated animals showed a mean of 23.03 g ($p<0.01$) weight loss as compared to HFD group, while

AEVF 300 mg/kg showed only preventive effect on weight gain. AEBP 300 mg/kg and 600 mg/kg treated animals showed a mean loss in body weight of 6.67 g ($p<0.01$) and 30.00 g ($p<0.001$) respectively. There were no significant changes observed in BMI in any of the treated groups except L-DOPA after treatment.

The high food intake indicates consumption of caloric diet. L-DOPA treated group showed a significant decrease in food intake ($p<0.01$) as compared to HFD group. All the test extracts AEMP, AEVF and AEBP also significantly reduced the food intake ($p<0.001$) as compared to HFD treated groups.

Accumulation of WAT is an important parameter observed in obesity. WAT mass of animals in L-DOPA, AEMP 400 mg/kg and AEBP 600 mg/kg treated animals showed significant reduction ($p<0.01$) whereas AEMP 200 mg/kg, AEVF 300 mg/kg and 600 mg/kg and AEBP 300 mg/kg showed no significant reduction in WAT mass as compared to disease control group. However they inhibited further accumulation of WAT mass.

Consumption of HFD is reported to increase plasma TG levels. In this study TG levels were lower in all treated groups as compared to the HFD group ($p<0.05$), but no significant changes were observed in TC and HDL levels.

The brain dopamine levels of HFD treated group was found to be low ($p<0.05$) as compared to normal diet control group. Treatment with L-DOPA and all plant extracts significantly improved brain dopamine concentration ($p<0.01$) as compared to HFD treated rats.

Conclusion

Treatment with L-DOPA as well as dopamine containing plants significantly lowered body weight and food intake in experimentally induced obesity in rats with corresponding significant increase in brain dopamine levels. Thus improvement in brain dopamine levels by herbal plants can be the probable mechanism for lowering of weight in obesity and can be used as a novel approach for studying effect of drugs for the treatment of obesity.

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My father has always remained as one of my best friends. Due to his wish and inspiration I have joined the PhD course. Till today at any moment I find myself depressed, the next moment I find him beside me.

Without my mother immense care and encouragement it would not have been possible for me to reach this stage. I am grateful to my wife for encouraging me to take up this endeavour. It would not have been possible for me to accomplish this task without their support and constant motivation. I cannot express in words the endless affection given by my kids, which were necessary to fuel my journey. It was their patience and jovial nature that made my long work hours at home possible.

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It will be really unfair if I don't mention about my innocent, sacrificed animals, if this project has been a success, it is just because of them only.

Javid I. Mansuri

Dedicated

To

My Beloved Family

Members

&

The Supreme God

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List of abbreviation

Abbreviations	Full form
L-DOPA	Levodopa
AEMP	Aqueous extract of *Mucuna pruriens* seeds
AEVF	Aqueous extract of *Vicia faba* seeds
AEBP	Aqueous extract of *Bauhinia purpurea* seeds
WHO	World Health Organisation
NFHS	National Family Health Survey
p.o.	Per oral
SD	Sprague Dawley
HFD	High fat diet
NPD	Normal protein diet
BMI	Body mass index
TC	Total cholesterol
TG	Triglyceride
HDL	High density lipoprotein
WAT	White adipose tissue
5-HT	5-hydroxytryptamine
SSRI	Selective serotonin Reuptake Inhibitors
SNRI	Selective noradrenaline Reuptake Inhibitors
MAOI	Monoamino oxidase inhibitors
HCL	Hydrochloric acid
EDTA	Ethylene diamino tetra acetate
CSIR	Council of Scientific & Industrial Research
NICE	National Institute for Health and Care Excellence
GLP-1	Glucagon-like peptide-1
EMA	European Medicines Agency
FDA	Food and Drug Administration

POMC	Pro-opiomelanocortin
RIO	Rimonabant in Obesity
5-HTP	5-hydroxytryptohane
TLC	Thin layer chromatography
HPTLC	High Performance Thin Layer Chromatography
SEM	Standard error of means
HbA1C	Glycated Haemoglobin Levels
ANOVA	One-way analysis of variance
IAEC	Institutional Animal Ethical Committee
DA	Dopamine
D2	Dopamine type 2 receptor
CT	Computed tomography
WHR	Waist-hip ratio
MRI	Magnetic resonance imaging
POMC	Pro-opiomelanocortin
SES	Socio-economic status
ARB	Angiotensin II receptor blocker
ACE	Angiotensin converting enzyme
ALLHAT	Antihypertensive Lipid Lowering Heart Attack Trial
RAAS	Ranin angiotensin aldosterone system
TNF- α	Tumor necrosis factor-α
IL-6	Interleukin-6
SNS	Sympathetic nervous system
ARC	Hypothalamic arcuate nucleus
AgRP	Agouti-related peptide
NPY	Neuropeptide Y
6-OHDA	6-hydroxydopamine
NA	Noradrenaline
DBS	Deep brain stimulation

CG	Cingulate gyrus
DLPFC	Dorsolateral prefrontal cortex
ANKK1	Ankyrin repeat and kinase domain containing 1
PET	Possitron enission tomography
DM	Diabetes mellitus
MCP-1	Monocyte Chemoattractant Protein-1
LDL	Low density lipoprotein
IAEC	Institutional Animal Ethics Committee

List of Symbols

%	Percentage
µ	Micro
µl	Microliter
A	Absorbance
º C	Degree Celsius
dl	Decilitre
g	Gram
h	Hour
IU	International Unit
Kg	Kilogram
m^2	Meter square
mg	Milligram
min	Minute
ml	Milliliter
cm	Centimeter
mm	Millimeter
nm	Nanometer
pg	Picogram
ng	Nanogram
w/v	Weight by volume
w/w	Weight by weight
wk	Weeks
rpm	Round per minute
M	Molar
v/v/v	Volume by volume by volume
ppm	Parts per million
AUC	Area under curve

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CHAPTER 1

Introduction

Obesity is a medical condition in which life is hindered by excess body fat. The generally accepted benchmark is the Body Mass Index (BMI). The prevalence of obesity is increasing not only in adults, but also among children and adolescents. The prevalence of obesity has increased steadily over the past five decades, and may have a significant impact on the quality adjusted life years (WHO guidelines, 2016). The causes of obesity may include dietary, exercise, social, cultural and financial factors. Sibutramine was widely marketed and prescribed as antiobesity drug until 2010, when it was withdrawn after a large study showed that it increased the risk of cardiovascular events and strokes and had minimal efficacy. An endocannabinoid receptor antagonist, rimonabant was withdrawn from the market due to concerns about its safety, including risk of seizures and suicidal tendencies. At present only one drug Orlistat has been approved for long-term use in the treatment of obesity. Orlistat promotes 5 to 10% loss of body weight and has its own limitations and side effects. This currently licensed drug is best, when used in combination with diet, exercise, and behaviour change regimens. However, it does not cure obesity and weight rebounds when discontinued. Some drugs employed to treat clinical obesity are associated with adverse effects such as nausea, insomnia, constipation, gastrointestinal problems, and potential adverse cardiovascular effects. Thus, there is a great demand for the search of new and safer anti-obesity medicines (Yanovski and Yanovski, 2014).

Several neurotransmitters (dopamine, norepinephrine and serotonin) as well as peptides and hormones like ghrelin are involved in the regulation of food intake (Gandhi and Swaminathan, 2017; Schwartz et al., 2000). Of particular interest is dopamine, since this neurotransmitter seems to regulate food intake (Martel and Fantino, 1996) by modulating food reward via the meso-limbic circuitry of the brain (Balcioglu and Wurtman, 1998). In a brain, nucleus accumbens is an important component of reward circuitry (Cone JJ et al., 2010) and the dopaminergic system is integral to reward-induced feeding behaviour (Wang

G et al., 2001).The influence of central dopamine signalling on feeding is thought to be mediated by the D_2 receptors (Geiger et al., 2007). Several lines of evidence support the hypothesis of altered dopamine function in obesity.

In fact, drugs that block dopamine D_2 receptors increase appetite and result in significant weight gain (Baptista, 1999; Wetterling, 2001) whereas drugs that increase brain dopamine are anorexigenic (Towell et al., 1988; Foltin, 1990; Wang et al., 1997). Additionally, an increase in body weight is a side effect of many commonly used drugs. Particularly, anti-dopaminergically acting neuroleptics, tricyclic antidepressants, lithium, and some anticonvulsants contribute to weight gain. Similarly, in obesity body mass index is negatively correlated with D_2 receptor density in the striatum (Wanget al., 2001; Haltia et al., 2007), which might reflect neuroadaptation secondary to over stimulation with palatable food (Colantuoni et al., 2001; Bello et al., 2002). Thus, increased food intake may be a compensatory behaviour for low dopaminergic drive (Paul and Paul, 2010; Daviset al., 2004). Recently it is reported that lower striatal activation in response to food intake was associated with obesity. Furthermore, this relation was modulated by genetically determined D_2 receptor availability (Inoue et al., 1995; Stice et al., 2008).

Many preclinical and clinical studies of important phytoconstituents of herbal plants have showed significant improvement in controlling body weight and food intake without any serious side effects.

Mucuna pruriens (L.) DC. (Leguminosae) known as ‘‘velvet bean” and ‘‘atmagupta’’ is a climbing legume, found in many parts of India, and South America. In the Ayurvedic system of medicine, *Mucuna pruriens* was used for the management of male infertility (Jayanthi, 2011), nervous disorders (Manyam et al., 2004) and also as an aphrodisiac (Kirtikar and Basu, 1996). *Mucuna pruriens* seed powder contains a large amount of L-DOPA (1.5%), which is a dopamine precursor and effective remedy for the relief in parkinson’s disease (Kasture et al., 2009). *Mucuna pruriens* seeds in addition to L-DOPA contain 5-hydroxytryptamine (5-HT), tryptamine, mucunine and mucunadine. Ethanolic extract of *Mucuna pruriens* shows protection against haloperidol induced tardive dyskinesia in rats (Dhanasekaran et al., 2010). *Mucuna pruriens* has been reported to inhibit chlorpromazine-induced hyperprolactinaemia in man (Dhanasekaran et al., 1978). *Mucuna pruriens* has proven to be more effective than L-DOPA in parkinson’s disease in animal model (Hussain and Gazala, 1997).It also shows anti-diabetic (Majekodunmietal.,

2011), anticancer (Rajeshwar at al., 2005), anti-oxidant (Sonpetkar et al., 2012), anti-hyperlipidemic (Eze et al., 2012) and protective effect against nephrotoxicity (Modi et al., 20008). These reports led us to hypothesize that high content of dopamine in *Mucuna pruriens* seeds could serve as a potent therapeutic agent for the obesity. However, their efficacy needs to be scientifically evaluated by *in vivo* experiments.

Vicia faba (Leguminosae) known as ‘‘broad bean” is widely grown and consumed, especially in China, North African countries and parts of Europe and North and South America, and is served in a large variety of forms, mostly based on the immature or mature seed (CSIR). *Vicia faba* seed powder contains a large amount of L-DOPA (0.75%) (Ingle, 2003), which is a dopamine precursor and effective remedy for the relief in parkinson’s disease (Spengos et al., 1988).*Vicia faba* seeds in addition to L-DOPA contain crude protein (27.5%), vicine and divicine (MdGolam e tal., 2009). Parkinson’s disease patients showed a significant improvement in their motor features after eating *Vicia faba* which was similar to the improvement measured after receiving 125 mg of levodopa plus 12.5 mg of carbidopa (Rabey et al., 1992). L-dopa of *V. faba* prolongs “On” period in patients with Parkinson’s disease who have ‘on-off’ effect fluctuation (Apaydin et al., 2000). It also shows anti-diabetic (Hussein et al., 2014), anti-inflammatory (Mohammed, 2012) and analgesic effect (Rajesh and Sunil, 2013).

Bauhinia purpurea is a shrub or small tree of Fabaceae family. It is found in most types of vegetation ranging from evergreen low lands, rain forests to mountain forests, up to 2000-3000 m altitude and also in savanna, scrub and dry deciduous forests to swamp forests on various soils. *B. purpurea* is good source of L-DOPA (1.34 %) (Vellingiri andHans, 2012). *Bauhinia purpurea* plant has been extensively used as Indian traditional and folklore medicine to cure various human ailments such as dropsy, pain, rheumatism, convulsions, wound healing, delirium and septicemia(Asolker et al., 2000; Ananth etal. 2010) and has analgesic, anti-inflammatory, nephroprotective activity (Shreedhara et al., 2009; Lakshmi et al., 2009) and antidiabetic activity (Muralikrishna etal., 2008). The alcoholic seed extracts shows anti-parkinson’s activity (Mopuri et al., 2010). The bark of the plant is used as an astringent and its decoctions are recommended for ulcers as a useful wash solution (Kirtikar and Basu, 2000). The leaves and roots are used for the treatment of catarrh, infection of children, boil and glandular swelling (Chakre etal., 2000). The aerial parts of the plant are reported to contain flavone glycosides, foliar flavonoids, 6-butyl-3-hydroxy

flavanone, amino acids, phenyl fatty ester, lutine and α-sitosterl (Yadava and Tripathi, 2000; Salantino and Blatt, 1999). These active constituents have been attributed in the therapeutic activity of the plant (Siedel et al., 1983).

In the light of above observations, current investigation was carried out to study the effect of dopamine containing plants *Mucuna pruriens* (L.) DC., *V. faba* and *B. purpurea* in HFD induced obesity on rats.

The study was conducted with following objectives:

1. Evaluation of anti-obesity effect of aqueous extract of *Mucuna pruriens* seeds on rats.
2. Evaluation of anti-obesity effect of aqueous extract of *Vicia faba* seeds on rats.
3. Evaluation of anti-obesity effect of aqueous extract of *Bauhinia purpurea* seeds on rats.
4. Comparison of the antiobesity effect of dopamine containing plants with the antiobesity effect of L-DOPA as a standard drug and precursor of Dopamine
5. To determine the correlation of the antiobesity effect of dopamine containing plants and L-DOPA with the brain Dopamine levels

CHAPTER 2

Literature Review

2.1 Obesity

Obesity is concerned with excessive overeating and preference for palatable, high-fat foods. It is characterised by excess adipose tissue accumulation where body mass index BMI ≥30 kg/m^2 (Sclafani, 2001; Gaillard et al., 2008).

2.1.1 Prevalence of obesity

- As per Global report released by WHO on 2014, there were more than 1.9 billion adults aged 18 years and older who were overweight. Of these over 600 million adults were obese.
- Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2014.
- 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight.
- Estimated 41 million children under the age of 5 years were overweight or obese.
- In Africa, the number of children who are overweight or obese has nearly doubled from 5.4 million in 1990 to 10.6 million in 2014.
- Nearly half of the children under 5 who were overweight or obese lived in Asia.
- Overweight and obesity are linked to more deaths worldwide than underweight.
- Globally there are more people who are obese than underweight – this occurs in every region except parts of sub-Saharan Africa and Asia (World Health Organisation, 2014).

2.1.2 Classification of obesity

BMI is a parameter to classify individuals as overweight or obese. It is calculated by body formula (weight (kg)/ height squared (m^2)). The guideline has been published by the World Health Organization (WHO) according to BMI (Table 2.1).

TABLE 2.1 Overweight and obesity according to BMI (WHO, 2000 and WHO, 2004)

Classification	**BMI (kg/m^2)**	
	Principal cut-off points	**Additional cut-off points**
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	18.50 - 24.99	18.50 - 22.99
		23.00 - 24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	≥30.00	≥30.00
Obese class I	30.00 - 34.99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

BMI measurement directly correlates the body fat with clinical condition and is a general method to determine adult obesity (Aronne, 2002). However, BMI measurement has some limitations as it does not consider some important factors like gender and age.

The risk associated with obesity depends on where fat is located. For example, individuals with peripheral obesity (fat located on the hip, thighs and buttoks) have fewer health risks

than those with central obesity (fat located around the stomach and gut). Other methods like waist-hip ratio (WHR) and waist circumference can be used to determine body fat distribution.

Computed tomography (CT) and magnetic resonance imaging (MRI) are the most precise methods for measuring abdominal obesity. However, they are expensive and impractical for clinical purpose.

Waist circumference measurement is a simple measure than WHR (Table 2.2). It can nevertheless predict increased intraabdominal fat as accurately as WHR (Lean ME et al., 1995).

TABLE 2.2 Waist circumference measurements and risk of obesity related complications

	Risk of Complications	
	Increased	**Substantially Increased**
Men	>94 cm/37 in	>102 cm/40 in
Women	>80 cm/32 in	>88 cm/35 in

2.1.3 Etiology of obesity

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been an increased intake of energy-dense foods that are high in fat; and an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing,

distribution, marketing, and education. The causes of obesity and its consequences are mentioned in Table 2.3.

TABLE 2.3 Causes of obesity

Primary causes	Genetic causes (Monogenetic disorders)	Melanocortin-4 receptor mutation Leptin deficiency POMC defficiency
Secondary causes	Neurological	Brain injury Brain tumor Consequences of cranial irradication
	Endocrine	Hypothyrodism Cushing syndrome GH deficiency Pseudohypoparathyrodism
	Psychological	Depression Eating disorders
	Drug induced	Tricyclic antidepressants Oral contraceptives

2.1.4 Risk factors for developing obesity

The risk of developing obesity for individuals varies and a number of risk factors have been identified, which include gender, age, ethinicity and socioeconomic status.

Gender: In general, women tend to have a higher prevalence of obesity, whereas the prevalence of overweight is greater for men. BMI of men compared to women is in higher percentage which is due to biological differences and women tending to deposit more body fat then lean tissue when they gain weight (James et al., 2001).

Age: An individual's risk of becoming obese varies over the life course. Children born into families where one or both parents are obese are more likely to become overweight or obese (Guillaume et al., 1995). According to one study, most overweight adults who had not been overweight as children and who were thin in children and adolescence were not protected from obesity as adults (Wright wt al., 2001). In addition they found that teenage overweight was a better predictor of adult obesity as overweight teenagers were. Women are more likely to become obese as compared to men during different stages in lifecourse: pregnancy, after childbirth, during the menopause and at retirement (Glenny et al., 1997).

Cross-sectional studies have demonstrated that obesity increases with age and most rapidly around twenties and early thirties and decline around late fifties (Rolland-cachera et al., 1991; Flegal et al., 1998).

Socio-economic status: There is an inverse relationship between obesity and socio-economic status (SES) particularly among women in both developed and developing countries. One report suggests that the risk factors for weight gain were low educational level (below college level) and consistently very low family income (Kahn et al., 2002).
A recent study found that women in low status employment were more likely to have a high BMI, a high WHR and were more likely to be overweight than women in high status employment- for example, professionals (Ball et al., 2002).
Education level is an indicator for SES and alternatively used to define income and many studies have demonstrated association between obesity and educational level. One study demonstrated that, lower education was associated with higher BMI in almost all female populations while women with higher education level were leaner than those with lower education (Molarius A, 2000). Although the strength of the inverse association between

obesity and SES in men appears to be inconclusive (Sobal and Stunkard, 1989). However few studies have reported that obesity is more common among both men and women in lower SES groups. Middle aged men, who had a high WHR were often unemployed and more likely to live in poorer housing conditions (Rosmond et al., 1996). In contrast men who had high status employment were more likely to be overweight (Ball et al., 2002).

2.1.5 Clinical manifestation/complications of obesity

Raised BMI is a major risk factor for many diseases such as cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2012 (Yusuf et al., 2004; Nanchahal et al., 2005), diabetes (Chanet al., 1994), musculoskeletal disorders (especially osteoarthritis – a highly disabling degenerative disease of the joints) and some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) (Benedetto et al., 2015; Vucenik and Stains, 2014; Mansuri et al., 2013). The brief outcomes of major risk factors of obesity are mentioned in Table 2.4.

TABLE 2.4 Health risks associated with obesity and its outcomes

Health risks associated with obesity	Outcomes
Metabolic syndrome' Type 2 diabetes Hypertension Hyperlipidemia	Coronary heart disease, Stroke Diabetes complications
Liver fat accumulation	Non-alcoholic steatohepatitis cirrhosis
Restricted ventilation	Exertional dyspnoea, Sleep apnoea Respiratory failure
Mechanical effects of weight	Urinary incontinence, Osteoarthritis Varicose veins
Increased peripheral steroid interconversion in adipose tissue	Hormone-dependent cancers like breast, uterus; Polycystic ovary syndrome (infertility, hirsutism)

Role of angiotensin II in obesity hypertension: Angiotensin II receptor blockade (ARB) or ACE inhibition inhibits sodium retention, volume expansion, and increased arterial pressure by inhibiting Angiotensin II stimulating sodium reabsorption, impairing renal-pressure natriuresis, and mediating hypertension in obese dogs (Hall JE et al., 1997; Robels RG et al., 1993). ARB also reduced blood pressure to a greater extent in obese-prone compared to obese resistant rats fed a high-fat diet. One study report suggests that in obese Zucker rats, the sensitivity of blood pressure reducing effect of angiotensin II is greater than lean rats (Alonso-Galicia M et al., 1996). The hypertensive effect induce in obese individuale is not clear whearther it is due to Angiotensin II or aldosterone secretion (Hall JE et al., 1999).

Some information can also be retrieved from retrospective analysis of the Antihypertensive Lipid Lowering Heart Attack Trial (ALLHAT) because there were many overweight and obese subjects in this trial and an ACE inhibitor was compared to other types of antihypertensive therapy (Oparil S, 2003). Obesity is also associated with RAAS activation induced glomerular injury and nephron loss. By constricting efferent arterioles, increased Angiotensin II formation exacerbates the rise in glomerular hydrostatic pressure caused by systemic arterial hypertension (Hall JE et al., 1999). Obesity with type 2 diabetic patients also clearly indicate that ARB or ACE inhibitors slow the progression of renal disease (Ravid M et al., 1996; Lewis EJ et al., 2001; Brenner BM et al., 2001). However, further studies are needed in nondiabetic obese subjects to determine the efficacy of RAAS blockers compared to other antihypertensive agents in treating hypertension and reducing the risk of renal injury.

Role of sympathetic nervous system in obesity-related hypertension: According to one study regarding obesity hypertension that correlate with increased SNS activity induced impairsment of natriuresis (Vaz M et al., 1997; Hall JE et al., Eslami P, Tuck M, 2003; Landsberg L, Krieger DR, 1989). Also assessment of elevated SNS activity done by microneurography and tissue catecholamines pillover especially in the kidneys and in skeletal muscle suggest obesity hypertension. It is also reported that hypertension in obese individual is controlled by inhibition of adrenergic activity than in lean subjects. Renal denervation markedly attenuates renal sodium retention and the development of obesity hypertension associated with a high-fat diet in experimental animals.

Mechanisms of SNS (Sympathetic Nervous System) activation in obesity: Number of mediators of SNS activation include angiotensin II (Ang II), hyperinsulinemia, activation of chemoreceptor-mediated reflexes associated with sleep apnea, impaired baroreceptor reflexes, increased levels of free fatty acids, and cytokines released from adipocytes (i.e.,"adipokines"), such as leptin, tumor necrosis factor-α (TNF- α), and interleukin-6 (IL-6) (Grassi G, 2000; Hall JE, 1993; Narkiewicz K et al., 1999; Wolk R et al., 2003).

Obesity and cancer: Many types of cancer, including esophageal, hepatocellular, breast, colon, endometrial, renal, and prostate cancer besides the profound effect on the manifestations of inflammation in many tissues and organs. The colon cancer was found in hyperinsulinemic obese patients.(Hall JE et al., 1995). The pathogenesis of colorectal cancer associated with diabetes, insulin resistance and increased BMI were also detrmined (Schoen RE et al., 1999). The risk of breast, esophageal, endothelial, and kidney cancer is on an average 20-33% associated with obesity (Caroll K et al., 1998). Mechanisms of cancer development or tumor growth include uncontrolled cellular proliferation, dedifferentiation and/or apoptosis, angiogenesis, and chronic adipokine-associated inflammation, along with the effects of cancer genes and/or environmental toxins that enhance inflammation. The Insulin-like growth factor-1 and leptin enhances cellular proliferation and/or dedifferentiation of adipose tissue adipokines and induce the cancer is a good example (Kim S and Popkin BM 1998; Campos JD, 2006).

Among men who did not initially have cancer and who were then followed for 16 years, those with BMIs of 30–34.9 had a 20% higher death rate from prostate cancer, whereas those with BMIs of 35–39.9 had a 34% higher death rate compared with men with normal BMIs (Calle EE et al., 2003). Although testosterone itself is a key prostate growth factor that may enhance cellular proliferation, it was speculated that enzymatic conversion of testosterone to estradiol within cells of benign prostatic hypertrophy caused them to dedifferentiate into prostate cancer cells (Massengill JC et al., 2003; Schatzl Get al., 2001; Freedland SJ, 2005). Obesity and advanced cancer except early prostate cancer are in good relationship was justify by many meta-analusis studies (Gong Z et al., 2006; MacInnis RJ and English DR, 2006).

Obesity is also linked with hepatocellular cancer that includes fatty liver formation which, after progressing from steatonecrosis to cirrhosis, becomes a risk factor for hepatocellular cancer. High leptin levels are also found in these obese patients and may be a growth-promoting factor for this cancer (Wang SN et al., 2006). Occurrence of pancreatic cancer in obese patient linked with inflammatory adipokines, which not only upset glucose transport, causing insulin resistance, but combined with hyperinsulinemia, hyperglycemia, and lipotoxicity, may lead to pancreatic β-cell inflammation and their exhaustion. It is speculated that the pancreatic dysplasia resulting from chronic inflammation associated with chronic pancreatitis promotes progression to pancreatic adenocarcinoma.

Multiple causes may contribute to both obesity and chronic inflammation, which are risk factors for esophageal carcinoma. It is supported by occurance of intestinal metaplasia in obese patient was associated with chronic inflammatory state induced Barrett esophagus. This may also be accentuated by chronic adipokine injury from visceral periesophageal adiposity appearing to enhance the progression of metaplasia to high-grade dysplasia, the premalignant precursor to esophageal carcinoma. Further complications of visceral adiposity include hiatal hernial formation and its associated decreased esophageal sphincter function, which, with increased abdominal pressure from visceral adiposity, further enhances gastric reflux (Chow WH et al., 1998). In addition, increases in the leptin levels seen in obesity may also contribute to cellular proliferation, dedifferentiation, and inhibition of apoptosis in this cancer (Ogunwobi O et al., 2006).

Adipokine secretagogues such as unbound insulin-like growth factor also enhance angiogenesis, which promotes cancer growth in general (Renehan AG, 2005). It was obsereved that decrese level of adiponectin, the adipocyte-secretory proteohormone, promotes the angiogenesis process to lead cancer in obese individuals (Brakenhielm E et al., 2004 and Cust AE et al., 2007). Incresed level of leptin have been found in many cancers like renal, esophageal, and hepatocellular carcinomas.

Obesity and sleep apnea: The prevalence of sleep disturbances rises dramatically in obese subjects (Vgontzas AN et al., 1994) and obesity is by far the most important modifiable risk factor for sleep disordered breathing (Bearpark H et al., 1995 and Young T et al., 2005). The difficulties like hypoventilation and breathing workload, respiratory muscle inefficiency, decreased functional reserve capacity and expiratory reserve volume, and

closure of peripheral lung units are gradually increased with obesity. These often result in a ventilation perfusion mismatch, especially in the supine position. Out of available treatment, weight loss in obese patients should always be advocated to improve sleep apnea (Vgontzas AN et al., 1994).

Obesity and type 2 diabetes: Obesity is an increased risk of developing insulin resistance and type 2 diabetes. In obese individuals, fat tissue releases increased amounts of glycerol, hormones, non-esterified fatty acids, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. Insulin resistance increases as body weight increases and therefore ability of insulin to transport blood glucose into fat and muscle. Weight reduction decreases insulin resistance (Steven E et al., 2006). Hyperinsulinemia, dyslipidaemia, and accelerated development of atherosclerosis are also associated with obesity. This combination of finding is commonly known as metabolic syndrome or syndrome X (Barrettt KE, 2010). The time dependent effects of obesity on diabetes have been clearly demonstrated (Table 2.5). When obesity persists for more than five years, the adjusted relative risk for developing diabetes is 8.7 compared to 4.9 if a person has been obese for less than five years (Wannamethee SG and Shaper GA, 1999).

TABLE 2.5 Relative risk of type 2 diabetes in obese patients (Wannamethee SG and Shaper GA, 1999)

	Relative risk of Type 2 diabetes	
BMI	**Subject in category >5 years**	**Subject in category <5 years**
>30	8	4.9
28-29.9	4.9	2.9
25-27.9	2.2	1.8
<25	0	1

2.1.6 Central mechanisms in body weight regulation

Hypothalamus of brain is the important part involved in the regulation of appetite is described in Fig 2.1 (Horvath TL, 2005). Regulation of food intake, energy consumption and expenditure and body weight is under the control of homeostatic process. The neural signals and gut signals are actually involved in food initiation and termination. The integration of these signals is coordinated by hypothalamic arcuate nucleus (ARC) of hypothalamus. In ARC two distinct group of neurones pro-opiomelanocortin (POMC) appetite-inhibiting neurons and the neuropeptide Y (NPY) and agouti-related peptide (AgRP) appetite-stimulating are coexisting. The signals from periphery results in alteration of relative activities and therefore feeding behaviour and energy utilisation (Schwartz MW et al., 1996; Meister B, 2000). Leptin and ghrelin have different effects on the hypothalamic neurones producing the various orexigenic and anorexigenic peptides, resulting in more or less opposing effects on energy balance (Klok MD et al., 2007).

Leptin: Leptin is a hormone that is secreted from white adipose tissue adipocyte in proportion to their fat mass. The Leptin acts on orexigenic NPY/AgRP neurones and inhibit the food intake while stimulate the anorexigenic POMC pathway which stimulate energy expenditure (Sahu A, 2004).

Ghrelin: Ghrelin has been shown to have appetite stimulating effects. Ghrelin attenuates leptin-induced reduction in food intake and body weight by modulating the expression of hypothalamic peptides. Ghrelin inhibits the effect of POMC neurones and CRH-producing neurones. On the other hand, it stimulates the activity of NPY, AgRP and orexin expressing neurones (Nakazato M et al., 2001; Kamegai J et al., 2001; Toshinai K, 2003)

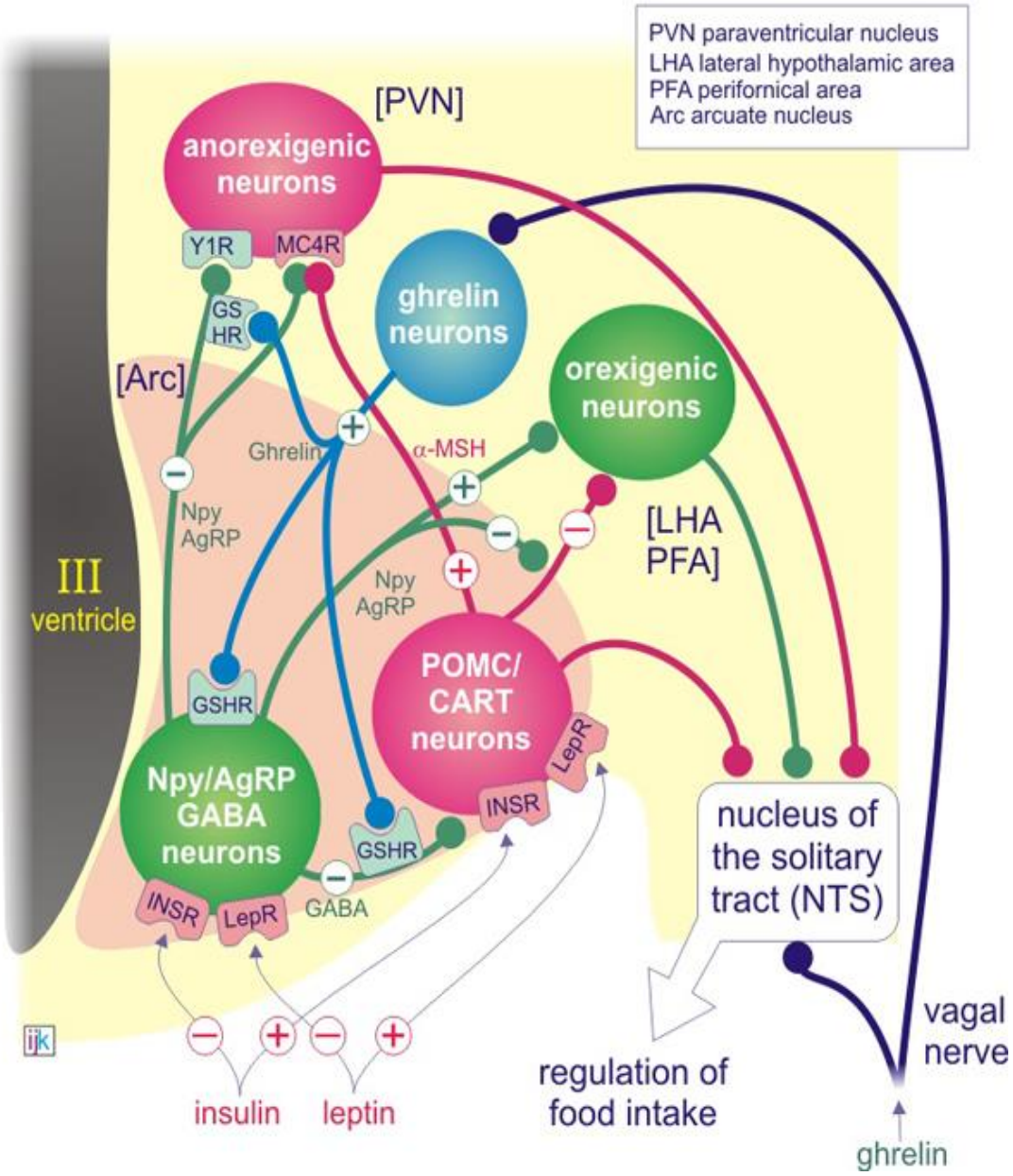

FIGURE 2.1 Hypothalamus and regulation of appetite

2.1.7 Brain Dopamine and Obesity

Reward related behaviours are mediated by DA system of the mesolimbic and mesocortical pathways. The role of dopamine in reward-related behaviors such as drug addiction and depression has received much attention because of severe consequences of dysfunction within the mesolimbic and mesocortical circuits. The DA-mediated food reward is associated to obesity is a major public health problem has been recently accepted.

Hypothalamus of the brain having homeostatic regulation center invoved in feeding behaviors and serves to integrate different hormonal and neuronal signals that control energy balance in controlling body weight. The body weight homeostatic mechanism is under the regulation of leptin, ghrelin and insulin like hormones (Morton GJ et al., 2006). However, the hedonic properties of food such as its sight, smell, and taste are strongly associated with reward and these hedonic qualities can override the homeostatic system (Palmiter RD et al., 2007). Therefore, delineating how this food reward circuit in the brain can control appetite and eating behaviors in connection with the brain's homeostatic system of energy balance is difficult.

Considerable evidence suggests that synaptic modifications of the mesolimbic DA system are critically associated with the rewarding effects of drugs of abuse as well as with food reward (Nestler EJ and Carlezon WA, 2006; Steketee JD and Kalivas PW, 2011; Kenny PJ, 2011). However, DA reward signaling is far more complex than it appears, and it is also implicated in learning and conditioning processes, as evidenced by studies revealing that dopaminergic reward signals are involved in coding for reward prediction error in behavioral learning (Schultz W, 1998; Schultz W, 2007; Schultz W, 2012; Wise RA, 2004).

In drug addiction, it is well known that the rewarding effects of drugs are primarily induced by increased DA release upon targeting of a specific substrate, such as the DA transporter in the case of cocaine. In food addiction, however, it remains to be elucidated how food reward can activate the DA reward signal in a manner similar to that evoked by drug addiction. It is important to understand the mechanisms by which these reward components induce adaptive changes in DA circuitry which is responsible for these addictive behaviors (Nestler EJ and Carlezon WA, 2006; Steketee JD and Kalivas PW, 2011; Kenny PJ, 2011).

Dopamine signalling in food reward: Drugs of abuse like cocaine may alter the dopaminergic mesolimbic system. The palatable food with high fat and sugar content demonstrate significant activation of dopamine reward circuitry. These findings suggest that food and drug addictions are depends on dopaminergic circuits which is a common neural substrates exist for both. It is also supported by human brain imaging studies of dopaminergic circuits in the regulation of food intake (Wang GJ, 2001; Small DM, et al., 2001; Small DM et al., 2003; Volkow ND et al., 2011).

It is also reported that the increases in synaptic DA concentrations in the mesolimbic system is trigger by abused drugs (Di Chiara Gand Imperato A, 1988). Likewise, it has been reported that

rewarding food stimulates dopaminergic transmission in the NAc (Bassareo V and Di Chiara G, 1997; Hernandez L and Hoebel BG, 1988; Roitman MF et al., 2004). DA measurement was done by microdialysis in the nucleus accumbens (NAc) of freely moving rats in the presence of food rewards, it was observed that amphetamine and cocaine injection increased DA levels in the NAc, which is normally activated by eating; thus, suggesting that the release of DA by eating could be a factor in food addiction (Hernandez L and Hoebel BG, 1988). Fast scan cyclic voltammetry at carbon fiber microelectrodes placed in the NAc of rats trained to press a lever for sucrose, the increase to respond for sucrose reward evoked by DA release in the NAc was obsereved (Roitman MF et al., 2004); this directly correlate the dopamine is a real-time modulator of food-seeking behavior.

There are also other studies revealed that dorsal striatum is invoved in the control of food reward rather than the NAc. It is supported by one example of cisflupenthixol injection (DA antagonist) into the dorsal striatum produces a suppression of food reward-associated lever pressing (Beninger RJ and Ranaldi R, 1993). Additionally, DA-deficient mice are hypophagic, and virally mediated restoration of DA production in DA-deficient mice reverses aphagia only when DA signaling in the caudate-putamen and dorsal striatum has been restored. In contrast, restoration of dopaminergic signaling to the NAc did not reverse aphagia, although the locomotor response to a novel environment or amphetamine was restored by viral delivery to the NAc (Beninger RJ and Ranaldi R, 1993; Szczypka MS, 2001).

In humans, mostly the dorsal striatum has been observed to correlate with feeding behaviors. For example, Small and coworkers used positron emission tomography (PET) on human subjects to show that regional cerebral blood flow measured while eating chocolate, correlated with pleasantness ratings in the dorsal caudate and putamen, but not in the NAc (Small DM., et al., 2001). One study of healthy human subjects, the PET images showed correlation between the reduction in DA ligand binding in the dorsal striatum and feeding (Small DM., et al., 2003). According to this finding, obese individuals have low striatal D2 receptor expression in proportion to their body mass index (Wang GJ, 2001).

D2 receptors in food reward: Although feeding increases extracellular DA concentration in the nucleus accumbens in rats, (Bassareo V and Di Chiara G, 1997; Hernandez L and Hoebel BG, 1988), as do drugs of abuse, DA depletion in the NAc in rats following bilateral injections of the neurotoxic agent 6-hydroxydopamine (6-OHDA) into the nucleus accumbens alone does not alter feeding (Salamone JD et al.. 1993). D1 and D2 receptors blockers in the NAc affects motor

behavior and the frequency and duration of feeding without reduction in amount of food consumed (Baldo BA et al., 2002). Another study reported that when exposed to the same high-fat diet, mice with lower D2 receptor density in the putamen gain more weight than mice with higher D2 receptor density (Huang XF et al., 2006), showing that the dopaminergic system responds to palatable food. According to one hypothesis, diet-induced obesity reduces mesolimbic DA function. The comparision of DA turnover in the mesolimbic DA system between rats fed a high-fat diet and those consuming a standard low-fat diet was done (Davis JF et al., 2008). The results demonstrated that animals consuming a high-fat diet, independent of the development of obesity, exhibited decreased DA turnover in the NAc, reduced preference for an amphetamine cue, and attenuated operant responses for sucrose.

The authors also observed that obesity induced due to a high-fat diet attenuated mesolimbic DA turnover in the nucleus accumbens, while there were no differences in DA turnover in the orbitofrontal cortex, suggesting a specific effect of a high-fat diet restricted to the NAc (Davis JF et al., 2008).

Deep brain stimulation (DBS) of the NAc shell was found to reduce binge eating and increased c-Fos levels in this region. It is justify by Raclopride, a DA D2 receptor antagonist induce attenuation of the effects of DBS, whereas the D1 receptor antagonist SCH-23390 was ineffective, suggesting that DA signaling involving D2 receptors is required for the effect of DBS in the NAc shell (Halpern CH et al., 2013). When they examined the effect of chronic NAc shell DBS in diet-induced obese mice, it was found to acutely reduce caloric intake and induce weight loss and, thus, supporting the involvement of D2 receptor-containing DA pathways in the food reward contributing to obesity, as well as the efficacy of NAc shell DBS in modulating this system (Halpern CH, et al., 2013).

A recent study showed a strong correlation between D2 receptor expression and compulsive eating behaviors (Johnson PM and Kenney PJ, 2010). It was observed that the body weight gain for given high dence and high palatable ‘cafeteria diet’ corelate compulsive eating behaviour (Johnson PM and Kenney PJ, 2010). Also it was suggested by decreased D2 receptor expression in the striatum with compulsive eating behaviour on obese rats. The body weight gain, increased fat mass, and hyperphagia is also demonstrated with selective deletion of insulin receptors in midbrain dopaminergic neurons in mice (Konner AC et al., 2011). Interestingly, in this group, DA

D2 receptor expression in the VTA was decreased as compared to that in the control group, suggesting a possible disinhibition of dopaminergic VTA/SN cells (Konner AC et al., 2011). However, it was also observed that compared to wild-type (WT) mice, D2 receptor KO mice have a lean phenotype and exhibit reduced food intake and body weight with enhanced hypothalamic leptin signaling (Kim KS et al., 2010). Acccording to these studies, the lacalization of D2 receptors in the brain leads to different outcomes, but we can not exactly prove the role of dopamine or DA D2 receptor in homeostatic regulation of enesgy balance as leptin, in addition to its role in food motivation behavior.

DA D2 receptors in human obesity: Many human studies have indicated the importance of the DA D2 receptor in regulating food reward in the context of obesity, particularly showing a change in striatal D2 receptor function and expression (Stice E et al., 2011; Salamone J D and Correa M, 2013). Obese people and drug addicts tend to show reduced expression of DA D2 receptors in striatal areas, and imaging studies have demonstrated that similar brain areas are activated by food-related and drug-related cues (Wang GJ et al., 2009; Volkow ND et al., 2009). PET studies suggest that the availability of DA D2 receptors is decreased in obese individuals in proportion to their body mass index (Wang GJ, 2001); thus, suggesting that DA deficiency in obese individuals may perpetuate pathological eating as a means to compensate for decreased activation of dopaminergic reward circuits. An alternative explanation is that individuals with low numbers of D2 receptors may be more vulnerable to addictive behaviors, including compulsive food in- take, and, thus, providing direct evidence of a deficit in DA D2 receptors in obese individuals (Wang GJ, 2001).

Based on the reduced D2 receptor availability in the striatal region of obese individuals, which suggests a possible role for D2 receptors in the inhibitory control of compulsive eating behaviors, Volkow and coworkers investigated whether D2 receptor availability in obese subjects would be associated with metabolism in prefrontal regions such as the cingulate gyrus (CG), dorsolateral prefrontal cortex (DLPFC), and orbitofrontal cortex, which are brain regions that have been implicated in various components of inhibitory control (Volkow ND et al., 2008). Their study revealed a significant association between D2 receptor levels in the striatum and the activity in the DLPFC, medial OFC, and CG in obese subjects. Since these brain regions are implicated in inhibitory control, salience attribution, and emotional re- activity, this finding suggests that disruption of these areas can cause impulsive and compulsive behaviors, and that

this may be one of the mechanisms by which the low D2 receptor levels in obesity contribute to over-eating and obesity (Volkow ND et al., 2008).

Interestingly, a recent report by Davis and coworkers demonstrated another aspect of the link between D2 receptor signals and compulsive eating behaviors (Davis C et al., 2012). They showed that obese adults with binge eating disorder differ biologically from their counterparts who do not binge eat. In fact, obese adults with binge eating disorder were characterized by a stronger DA signal when compared to their obese but non-binging counterparts, a difference that was associated with a distinct genetic polymorphism of TaqIA of the D2 receptor gene (Davis C et al., 2012).

Recently reported one study focus positive relationship between body mass and D2/D3 receptor agonist binding effect in the ventral striatum (NAc) of non-obese subject, but no relationship was found with antagonist binding. These data demonstrate an association of high body mass with increased D2 receptor affinity in the NAc, and their motivation to consumes more palatable foods (Caravaggio F et al., 2013)..

It is necessary to determine expression of D2 recptor and its signalling mechanism to correlate the body weight reducing effect in humans (Martinez D et al., 2004).

2.1.8 Pathophysiology of obesity

Palatable and high-fat diets can lead to obesity with or without hyperphagia. Increased inflammatory, mitochondrial, and oxidative stress signalling within the brain leads to neural/microglial alterations impinging on the hypothalamus and mesocortico-mesolimbic pathways involved in reward functions, accelerating the development of obesity (Redinger, 2007; Carlin et al., 2013). The pathophysiology of obesity is mentioned in Fig. 2.2.

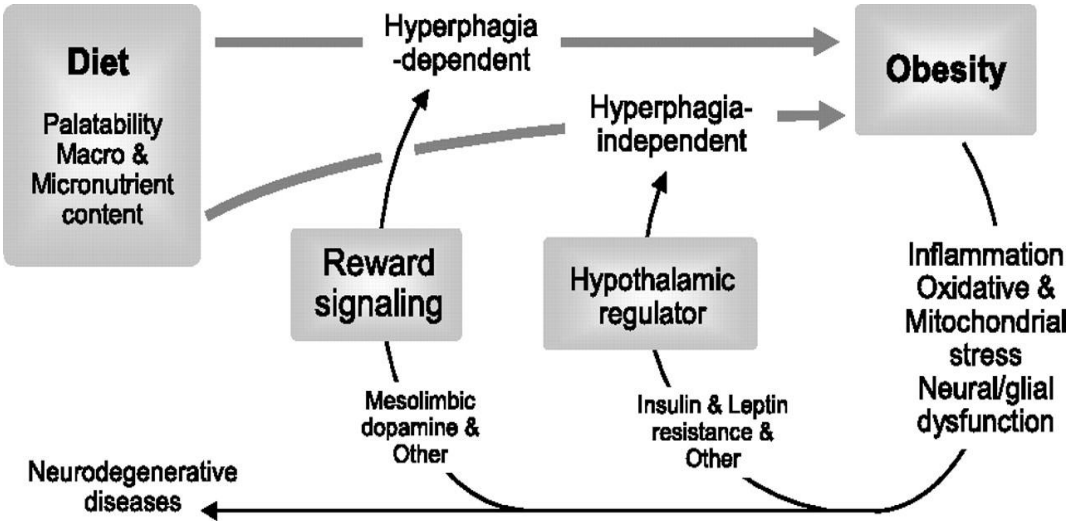

FIGURE 2.2 Reward circuitry and hypothalamic energy balance regulator in pathophysiology of obesity

2.1.9 Currant treatment of obesity

Drug therapy may be a suitable option for people who have been unsuccessful in losing weight with lifestyle changes. Patients should use medicines in addition to appropriate diet, physical activity and behavioural interventions (Haslam, 2015).

Orlistat is a pancreatic lipase inhibitor that causes inhibition of digestion of dietary fat and increases excretion of around 30% unabsorbed fat in the faeces (Yanovski SZ and Yanovski JA, 2014). It is available at two different doses of 120 mg and 60mg. It is safe for long-term use; although weight loss is around 3-4% (Sjostrom et al., 1998). Adverse effects of orlistat are common like abdominal cramps, oily stools and occasional faecal incontinence. Risk improvement includes reducing LDL cholesterol by 5-10%, lowering blood pressure and HbA1c (Davidson et al., 1999). It also reduces the cumulative incidence of diabetes over four years of 37.3% in obese normoglycaemic subjects (Torgerson et al., 2004).

The National Institute for Health and Care Excellence (NICE) encourage the use of Orlistat in individuals with BMI >30, or >28 with co-morbidities. Orlistat should only be continued for more than three months if the person has lost minimum 5% of their initial body weight since starting drug treatment (NICE, 2010).

The use of drug for more than 12 months as a maintenance of weight should be decided after discussing with the patient about their potential benefits and limitations.

Liraglutide is a class of glucagon-like peptide-1 (GLP-1) receptor agonist and has been used as a glucose-lowering agent in diabetes. It is administered s.c. daily. It has recently been given a positive opinion by the European Medicines Agency (EMA) and is authorised by the US Food and Drug Administration (FDA) as an anti-obesity agent. The loss of appetite is induced by feeling of fullness of stomach with corresponding inhibiting hunger signals. The EMA recommends to priscribed Liraglutide who BMI>30, or 27 with associated type 2 diabetes especially adult population. The 3 mg daily dose is recommanded. The treatment should be discontinued if body weight has not been reduced by at least 5% after 12 weeks.

Liraglutide at a dose 1.2 mg and 1.8 mg has shown to produce >5% weight loss during trials in diabetic patient as reported by the LEAD series of trials (Niswender et al., 2013). The SCALE programme has also assessed liraglutide for weight loss in obese patients with or without type 2 diabetes at a higher dose of 3 mg daily. During SCALE maintenance trial, patients who lost at least 5% body weight (up to 6.3kg) in a 12-week intensive diet run-in period were exposed to 3mg liraglutide or placebo daily for 56 weeks. After 56 weeks, 81% of liraglutide-treated patients maintained the weight loss (Wadden et al., 2013).

Lorcaserin is selective 5HT2C receptor agonist. Weight loss by Lorcaserin is due to increases satiety. It has been shown to cause weight loss of 3–4kg at one year, alongside improvements in fasting blood glucose, insulin sensitivity, heart rate, total blood pressure, LDL cholesterol and C-reactive protein levels (Chan et al., 2013).

Diethylpropion and phentermine are sympathomimetic amines, which reduce appetite by increasing noradrenaline release (Li et al., 2005). This has been shown to result in a placebo-subtracted weight loss of 7-8 kg at 36 weeks (Kang, 2010). The adverse effects include sympathetic side effects, such as hypertension, tachycardia, insomnia, dry mouth, constipation and nervousness, which require close monitoring without drug addiction or abuse liability.

Phentermine in combination with Topiramate as Qsymia in USA in 2012 was launched initially, and induces a placebo-adjusted weight loss of up to 10% of body weight (8-9 kg at one year) (Allison et al., 2012). Alongside risk-factor improvement, included an almost

80% reduction in cumulative incidence of diabetes in an impaired glucose tolerant population, increased HDL, and reduced HbA1c, LDL and blood pressure. Clinical trials include CONQUER, EQUIP, and SEQUEL that led to its approval (Garvey et al., 2012). CONQUER was a 56-weeks, randomised, double-blind, placebo-controlled, phase III trial in which two Qsymia treatment arms and one placebo arm were initiated in conjunction with a 500 calorie per day dietary deficit. After one year of treatment, a reduction in body weight of 12.9 kg with phentermine 15mg plus topiramate 92 mg, and 9.9 kg with phentermine 7.5 mg plus topiramate 46mg was observed compared with 1.8 kg in the placebo group (Gadde et al., 2011).

Contrave, a combination of dopamine-reuptake inhibitor bupropion with opioid antagonist naltrexone, was licensed in the US in 2014 (Wadden et al., 2011). The mechanism of Bupropion is to stimulate the POMC neuron, which plays a role in the regulation of appetite, while naltrexone prevents inhibition of POMC neurons by blocking the action of ß-endorphin, thus prolonging the effect of bupropion.
More obesity drugs have been withdrawn than licensed by European authorities over recent years.

Withdrawn weight management drugs

Rimonabant was an endocannabinoid receptor blocker. The data of Rimonabant in Obesity (RIO) trials showed loss of around 8.5-9 kg body weight in subjects (Van Gaal et al., 2005). Furthermore, it induced a reduction in HbA1c of up to 1.9% in diabetes (Rosenstock et al., 2008). The depressive mood changes were observed during initial phase of Rimonabent. Later on licence of Rimonabent was withdawn from market because of persistent links with suicidality (Thomas et al., 2014).

Sibutramine was initially approved for weight management as its satiety enhancer effect has been found in clinical trials (Cheung et al., 2013). Sibutramine increases synaptic concentration of 5-HT, NE and Dopamine via reuptake inhibition. Patients taking sibutramine required closed monitoring over blood pressure every two weeks, and any rise resulted in cessation of treatment. It was also contraindicated in high-risk patients with cardiovascular disease. Dry mouth, anorexia, constipation, appetite increase, insomnia, dizziness and nausea were noted more frequently in sibutramine treated subjects.

Treatment was licensed for one year and stopped early in patients who did not respond to treatment.

As part of the licensing commitments, a cardiovascular safety trial SCOUT (James et al., 2010) was carried out. The study were carried out in high-risk patients with cardiovascular disease and diabetes and patients were required to take sibutramine for five years, regardless of whether they responded by losing weight. Cardiovascular side effects put a question mark on their use. This caused the withdrawal of the drug, based on the assumption that anyone who is obese potentially has cardiovascular disease, despite no adverse trends having been noted in the licensed population. However, post-hoc analysis showed that mortality would have been reduced if the SCOUT cohort had been given sibutramine in line with its licence (Caterson et al., 2012).

2.1.10 Recent advances in anti-obesity drugs

TABLE 2.6 List of drugs under an investigational phase (Rodgers, RJ et al., 2012).

Name or code	Class	Status
Cetilistat	Pancreatic lipase inhibitor	Phase III
TM30339	Neuropeptide Y5 receptor antagonist	Phase II
Emaglutide	GLP-1 receptor agonist	Phase III (DM) Phase II (obese)
Remogliflozin etabonate	SGLT-2 inhibitor	Phase II (DM) Phase I (obese)
GT389-225	Conjugate of pancreatic lipase inhibitor & fat binding polymer	Phase I
BVT. 74316, PRX 07034	5HT-6 receptor antagonist	Phase I
TKS1225	GLP-1 receptor agonist	Phase I
Tesofensine	5-HT/DA/NA reuptake blocker	Phase III
Amylin analogue	Amylinomimetic	Phase I
KRP-204	Selective β-3 adrenoceptor agonist	Phase II
Obineptide	Neuropeptide Y2 + Y4 receptor agonist	Phase II
SLx-4090	Mitochondrial transfer protein inhibitor	Phase II

2.1.11 Surgical treatment of obesity

Bariatric surgery

Bariatric surgical techniques are divided into two groups:

1) Malabsorptive
2) Restrictive procedures (Marielle et al., 2008).

Malabsorptive procedures induce malabsorption of nutrients by shortening the functional length of the small intestine (Schneider BE and Mun EC, 2005). The short-bowel induces negative energy balance and weight loss. The jejunoileal bypass was one of the first bariatric operations. The long-term complications include malnutrition, electrolyte imbalances, vitamin deficiencies, liver failure, renal (oxalate) stones, and death. Therefore, this procedure is no longer advisable (Santry HP et al., 2005).

Biliopancreatic diversion and the biliopancreatic diversion with duodenal switch are the two different malabsorptive techniques used currently (Schneider BE and Mun EC, 2005; Santry HP et al., 2005; Buchwald H, 2004). The mechanism of these techniques is to perform a partial gastrectomy. The biliopancreatic diversiontechnique include horizontal distal gastrectomy with a gastro-ileostomy and gastro-jejunostomy; this long (food) limb is anastomosed to the biliopancreatic (bile, a pancreatic juice) limb (Schneider BE and Mun EC, 2005; Buchwald H, 2004). In a biliopancreatic diversion with duodenal switch, a pylorus-sparing sleeve gastrectomy with duodeno-ileostomy is performed (Schneider BE and Mun EC, 2005; Buchwald H, 2004). It is generally accepted that biliopancreatic diversion with duodenal switch results in less cases of dumping and marginal ulcers than a classical biliopancreatic diversion (Hess DS and Marceau Pet al., 1998). In both procedures, the length of the common limb – i.e., the time during which digestion and nutrient absorption can occur – determines the degree of malabsorption (Schneider BE and Mun EC, 2005; Buchwald H, 2004).

Restrictive operations induce satiety by reducing the storage capacity of the stomach leading to a decreased caloric intake (Schneider BE and Mun EC, 2005). Restrictive procedures are comparatively simpler to perform than malabsorptive procedures because less chances of complications during procedure. The most common tchniques of restrictive

procedures include vertical banded gastroplasty and laparoscopic adjustable gastric band technique.

In vertical banded gastroplasty techniques, the fundus of the stomach is stapled parallel to the lesser curve using a surgical stapling device. The distal exit of the created pouch is narrowed with a band. A food-receiving reservoir of 50 ml remains and the banding provides an outlet diameter of 10–12 mm (Schneider BE and Mun EC, 2005; Buchwald H, 2004).

In laparoscopic adjustable gastric band technique, a silicon inflatable gastric band is placed horizntallly around the proximal part of the stomach. A pouch is created by inflating the gastric band via s.c. port. As patient point of view the diameter of the band is adjustable. (Schneider BE and Mun EC, 2005; Buchwald H, 2004).

The weight reduction can also be achieved by placing an intragastric balloon filled with saline in the gastric cavity. It is used as a temporary method for such type of obese patient who refuse bariatric surgery (Spyropoulos C et al., 2007; Maggard MA et al., 2005). In the United States, the most commonly performed bariatric procedure is the Roux-en-Y gastric bypass. It incorporates both aspects, restrictive as well as malabsorptive. In this method a gastric pouch is created and separated from the remainder of the stomach. The satiety is induce by connecting Roux-Y-limb of stomach to the jejunum and during the eating the gastric pouce very fastly fills and food material pass into the jejunum. (Schneider BE and Mun EC, 2005; Buchwald H, 2004).

2.2 Medicinal plants in obesity

The available approved anti-obesity drugs in market are very less as many drugs were withdrawn from the market due to serious side effects. The medicinal plants for treatment of obesity have been explored extensively in the present scenario. Some important medicinal plants and their respective bioactive compounds have been identified and there effectiveness in treament of obesity was studied.

The important medicinal plants containing active phytoconstituents and their mechanism of action in treatment of obesity in animals are mentioned below (Table 2.7). Table 2.8

shows some plants activity against obesity by study on cell lines. The plant tested on human volunteers or clinical trails are listed in Table 2.9. Anti-obesity activity in isolated cell organelles and isolated cellular enzymes specifically pancreatic lipase are listed in Table 2.10 and 2.11 respectively (Patra S et al., 2015).

TABLE 2.7 Anti-obesity effects of plants with mechanism of action studied on animal models

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
1	*Angelica gigas* Nakai (Apiaceae)	Roots	Inhibition of adipocytokines such as leptin, resistin, IL-6 and MCP-1 secretion.	HFD induced obesity in mice
2	*Argyreia nervosa* Bojer (Convolvulaceae)	Roots	Reduction of leptin, total cholestrol, LDL, and triglycerides levels	Diet-induced obesity in rats
3	*Atractylodes lancea* (Thunb.) DC (Compositae)	Rhizome	Inhibition of pancreatic lipase	High-fat diet-induced obesity in mice
4	*Bauhinia variegate* L. (Fabaceae)	stem, roots, stem bark	β-sitosterol in the stem induces secretion of serotonin in brain exhibits anti-obesity activity.It reduces increased level of total cholesterol, triglycerides, LDLP and increases the level of HDLP, brain serotonin level.	High caloric diet
5	*Cordia ecalyculata* Vell (Boraginaceae)	Whole plant	Centrally promoting action of adrenaline	
6	*Clerodendrum phlomidis* L. f. (Lamiaceae)	Roots	β-sitosterol mediated inhibition of pancreatic lipase enzyme.	High fat diet induced obesity in C57BL/6J mice
7	*Glycine max* (L.)	Bean	The extract decreases appetite	Diet-induced obese

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
	Merr. (Leguminosae)		and HFD-induced body weight gain through leptin-like STAT3 phosphorylation and AMPK activation.	mice
8	*Ligularia fischeri* (Ledeb.) Turcz. (Compositae)	Leaves	Inhibition of pancreatic lipase by polyphenols	C57BL/6 mice
9	*Lithocarpus polystachyus* (Wall. ex A.DC.) Rehder (Fagaceae).	Leaves	Reduces serum leptin and insulin levels, decreases lipids levels, anti-oxidant property, raise serum adiponectin, reduce circulating CRP and and inhibit expression of PPARγ and C/EBPα.	High fat diet-induced obese rats
10	*Morus australis* Poir (Moraceae)	Fruit	Reduces serum leptin and insulin levels	Male C57BL/6 mice fed with high-fat diet
11	*Rosmarinus officinalis* L. (Lamiaceae)	Leaves	Extract decreases leptin, tumor necrosis factor alpha, IL-1β. The extract also reduces serum triglycerides, cholesterol and insulin levels.	Lean (Le,*fa*/+) and obese (Ob,*fa*/*fa*) female Zucker rats
12	*Ziziphus mauritiana* Lam (Rhamnaceae)	Bark	It supresses, reduces body weight and fat accumulation	High Fat Diet induced obesity in Wistar rats

TABLE 2.8 Anti-obesity effects of plants with mechanism of action studied on cell line as model

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
1	*Dalbergia sissoo* DC. (Leguminosae)	Leaves	Anti-obesity effect by inhibiting pancreatic lipase activity	Pancreas of chicken

TABLE 2.9 List of plants exhibiting anti-obesity activity studied on human volunteers

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
1	*Carum carvi* L. (Apiaceae)	Seed	Reduction of Body weight and BMI, fat mass and waist-to-hip ratio.	Human clinical trials
2	*Cissus quadrangularis* L. (Vitaceae)	Cylaris a formula contains a *C.quadrangulare* xtract	Improve serotonin levels, Also pancreatic lipase inhibitory action, reduce the absorption of dietary fats by phytosterols	Human clinical trials
3	*Citrus aurantium* L. (Rutaceae)	Fruits, leaves	Down-regulattion of expression of C/EBPβ and subsequently inhibits the activation of PPARγ and C/EBPα. Also increses energy expenditure and weight loss by active constituent p-synephrine	Human clinical trials
4	*Garcinia cowa* Roxb. ex Choisy (Clusiaceae)	Fruit	Weight loss mediated be inhibiting ATP-dependent citrate lyase enzyme	Human clinical trials

5	*Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae)	Leaves	Inhibit adipogenesis by downregulating PPARc and CEBP1a as well as lipogenic factors including fatty acid binding protein 4 and lipoprotein lipase.	Human clinical trials
6	*Nigella sativa* L. (Ranunculaceae)	Commercial *Nigella sativa* oil prepared by steam distillation.	Antihyperlipidemic action	Human Volunteer
7	*Salacia reticulate* wight (Celastraceae)	Root	Body weight, fat and BMI reduction	Clinical trials
8	*Trigonella foenum-graecum* L. (Leguminosae)	Seed	Decrease daily food consumption and increases energy expenditure. Also insulin/glucose ratio decreases significantly.	Clinical trials
9	*Turnera diffusa* Willd. ex Schult. (Passifloraceae)	Leaves	Delays gastric emptying, reducing the time to perceived gastric fullness and induces weight loss.	Healthy volunteers
10	*Vernonia amygdalina* Delile (Compositae)	Leaf	Fat mass reduction	Clinical trials
11	*Ziziphus jujube* Mill. (Rhamnaceae)	Fruit	Inhibits lipid accumulation by reducing expression of adipogenic transcription factor like PPAR-γ	Clinical trials

TABLE 2.10 Anti-obesity effects of plants with mechanism of action studied on isolated cell organelles

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
1.	*Verbesina persicifolia* DC (Compositae)	Aerial parts	Increases energy expenduture	Rat liver mitochondria

TABLE 2.11: Anti-obesity effect of plants with mechanism of action studied on isolated cell enzymes

Sr No.	Plant name	Part(s)	Mechanism	Experimental model
1.	*Fraxinus chinensis* subsp. rhynchophylla (Hance) A.E.Murray (Oleaceae)	Stems and barks	Inhibition of pancreatic lipase enzyme by phytoconstituents like secoiridoids ligstroside, oleuropein, 2''-hydroxyoleuropein and hydroxyframoside B.	Porcine pancreatic lipase
2	*Vitis vinifera* L. (Vitaceae)	Seed flowers, peel, roots, fruit	Inhibits lipid accumulation by reducing expression of adipogenic transcription factor like PPAR-γ.	Pure pancreatic lipase

2.3 The Plant: *Mucuna pruriens*

FIGURE 2.3 *Mucuna pruriens* seeds and pods

2.3.1 General profile

- Plant Name: *Mucuna pruriens*
- Family: Leguminosae
- Geographical Distribution: Wide spread in tropical and sub-tropical regions of the world. It is found in bushes and hedges at damp places, ravines and scrub jungles throughout the plains of India. It is cultivated for its pods as vegeTABLE and young leaves as fodder. It is cultivated in Bangladesh, India, Sri Lanka, South East Asia and Malaysia
- Common Names: cowhage, ‘‘velvet bean”, ‘‘atmagupta’’ (Kirtikar KR et al., 1996).

2.3.2 Cultivation

Mucuna is a popular kharif crop in India. Seeds are sown at rate of 50 kg/ha between 15 June to 15th July with plant spacing of 60 × 60 cm. Delayed sowing may result in infestation of aphids (Oudhia P, 2001a).

Although, no named cultivar of Mucuna is available, locally available seeds possess good viability and higher germination (Oudhia P). Plant support increases yield by 25%

and reduces pest infestation. Normally flowering begins 45-50 days after sowing (Oudhia P and Tripathi RS, 2001). Yields of 5000 kg/ha have been recorded from well managed irrigated crop having supports (Farooqi AA et al, 1999and Singh BM et al., 1995).

2.3.3 Macroscopic description

Table 2.12 Macroscopic description of *Mucuna pruriens* plant

Part(s)	Description
Leaves	• Fairly large, alternate, stipulate and pinnately trifoliate, • Stipels minute and osculate • Leaflets are 3-4 inch long and 2-3 inch wide
Flowers	• Short stalked, large, dark or lurid purple, turning dark when dry, with bracts and bracteoles • Pedicels are short, $1/8^{th}$ to $1/4^{th}$ inch and densely clothed with sharp hairs, shorter than the calyx, • Calyx are gamosepalous, about 3/4 of inch long, covered outside with whitish silky hairs intermixed with a few irritant bristles • Corolla are papilionaceous much exerted, purplish • Anthers are alternately oblong and ovate, round. • Ovary are sessile, villous, many ovuled with a faliform incurved style hairy below and ending in a capitates stigma
Fruits	• The fruit contains 4-6 or more seeds with septa or partitions between the seeds • Seeds are ovoid or transversely oblong slightly internally compressed with a polished dark brownish or black or occasionally mottled testa • These are 2-3 or 4 inch long and half an inch broad turgid explosively dehiscing pod, slightly covered at both ends.
Root	• The roots consist of many long, but softly woody, somewhat flexible roots, having a diameter of 7 mm or more • The outer surface is dark brown to black in colour and slightly

	rough due to the presence of many oblong slightly protruding prominent lenticels and a few rootlets
Seeds	• Seeds are black in colour, reniform in shape, 15-20 mm long and 7-15 mm broad • Funicular helium and cellular pit growth around the helium. • Seed coat is hard, thick and glossy occasionally mottled. • Embryo fills up the seed and is made up of two large cotyledons.

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2.3.4 Microscopic description

Table 2.13 Microscopic description of *Mucuna pruriens* plant

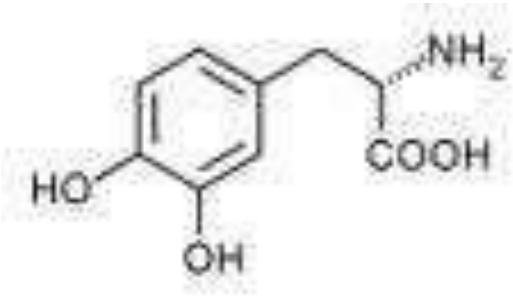
Part(s)	Description
Epidermal features	• The epidermis consists of single layer of cells • Unicellular trichomes are found on both the surfaces • A well-developed cuticle is present on both the surfaces of the leaf • The leaves having stomata are anomocytic. The number of stomata per unit area is always higher on the lower surfaces than on the upper surfaces. Trichomes are present in a great number on adaxial or abaxial surfaces of the leaf. The trichome frequency is more on vein than in the intercostal region. Unicellular trichomes are composed of thick walled long, narrow cells(Dhale DA, 2010).
Petiole	• Sectional views of petiole shows more or less circular in shape. Epidermis consists of barrel shaped cells, cell walls thick with outside thick cuticle. • Hypodermis is collenchymatous with 2-3 layered followed by parenchymatous cortex. • Vascular bundles are of dictyostele type and occur scattered within cortex in circular manner. • Phloem consists of sieve tubes, companion cells and phloem parenchyma also.

	• Xylem consists of vessels, tracheids and xylem parenchyma. Oxalate crystals occur in the cortex (Dhale DA, 2010).
Leaf	• Dorsiventral, hypostomatic. • Upper epidermal cells are large. • Lower epidermis cells are smaller. • On both surfaces tomata are present. • Papillae absent. • Tricomes are non-glandular and unicellular • The mesophyll is differentiated into palisade and spongy tissue. Palisade is 1-2 layered and spongy tissues are of 4-6 layered. • Vascular bundle isan arc shaped • Xylem element facing upwards. • Phloem have sieve tubes, companion cells and phloem parenchyma • Xylem have vessels, tracheids and xylem parenchyma. • The epidermis in midrib region is followed by 2-3 layered collenchymas on abaxial surface and one layer of collenchyma on adaxial surface. • The collenchymatus hypodermis is followed by parenchymatous cortex in which vascular bundle is present.
Stem	• The stem has an irregular outline. Epidermis has small cells compactly arranged; Bellow the epidermis a 4-5 layers band of collenchyma and 3-4 layers a zone of parenchyma are precence. • The stem possesses a dictiostele which encloses wide central pith. Phloem consists of sieve tubes, companion cells and phloem parenchyma • Xylem consists of vessels, tracheids and xylem parenchyma. • Starch grains and calcium oxalate crystals of acicular type are found abundantly in the region of the stem.
Root	• The cork consists of 4 to 6 rows of nearly cubical to rectangular cells of which, the cells of the peripheral rows are thick walled, but

	not lignified while the innermost one or two rows are thin walled. Phellogen is single layered. • The cortex is wide zone consisting of tangentially elongated cells and small group of stone cells. • Starch grains of simple type are present in the cortex • Each phloem group is composed of narrow tangential strips of phloem fibers alternating with the thin-walled phloem-elements. • Xylem is developed in large amount with linear manner (Dhale DA, 2010).
Node	• A solitary arc shaped vascular strand is emerged out from the stele at the nodal region. • A single gap is formed. • The node is thus unilacunar one-traced.

2.3.5 Phytoconstituents

- L-DOPA (1.5 %), a potent neurotransmitter precursor of dopamine (Damodaran M et al., 1937; Modi KP et al., 2008; Paresh BS et al., 2010).
- Tryptophane, 5-hydroxytryptohane (5-HTP) (Tripathi and Upadhyay, 2001).
- Mucunine, mucunadine, prurienine and prurieninine.
- Fatty content (Ghosal S et al, 1970).
- Oils like palmitic, oleic, stearic and linoleic acids
- Amino acids like lecithin, gallic acid, glutathione and beta-sitosterol.



Structure of L-DOPA

2.3.6 Pharmacological studies

Anti-parkinson's and neuroprotective effect

- Extract of *Mucuna pruriens* seeds has shown anti-parkinson's and neuroprotective effect on rodent models (Kasture SG et al., 2009; Manyam BV, 2004).
- *Mucuna pruriens* has proven to be more effective than L-DOPA in parkinson's disease in animal model (Hussain G et al., 1997).
- The ethanolic extract of *Mucuna pruriens* has shown protection against haloperidol induced tardive dyskinesia in rats (Dhanasekaran S et al., 2010).
- Clinical effectiveness of mucuna preparation (15 and 30 g) were compared with single doses of 200/50 mg L-DOPA/Carbidopa in randomised order at weekly intervals. It was found that compared with standard L-DOPA/Carbidopa, the 30 g mucuna preparation has considerably faster onset of effect. The rapid onset of action and longer on time without concomitant increase in dyskinesias in patients on mucuna seed powder formulation suggest that this natural source of L-DOPA might possess advantages over conventional L-DOPA preparations in the long term management of Parkinson's disease (Katzenschlager R, 2004).

Anti-fertility effect

- The extract of *Mucuna pruriens* seeds powder is found to treat infertility in male albino rats (Jayanthi A, 2011).
- The ethanolic extract of *Mucuna pruriens* at 200 mg/kg dose produced significant increase in the sexual activity of normal rats as compared to the control (Suresh et al., 2009).
- The antifertility effect of (5 g /day, p.o.) was studied on 60 subjects. The semen samples were collected before treatment and after 3 months of treatment. The treatment subjects showed significant improvement in sperm count and motility. *Mucuna pruriens* seed powder also restored the levels of GSH, SOD, catalase and ascorbic acid in seminal plasma of infertile men (Shukla KK and Mahdi AA, 2010).

Antidiabetic effect

- The ethanolic extract of *Mucuna pruriens* at 100 mg/kg dose shows 55.4% reduction in glucose levels of the diabetic rats in alloxan induced diabetes on rats (Majekodunmietal, 2011).
- The aqueous extract of *Mucuna pruriens* (100 and 200 mg/kg body weight) significantly reduced blood glucose level from 127.5±3. 2 to 75.6±4.8 mg % after an oral glucose load (Bhaskar A et al., 2008).

Anticancer activity

The methanolic extract of *M. pruriens* has been shown to have anticancer activity against Ehrlich ascites carcinoma in Swiss albino mice at a dose of 125 mg/kg and 250 mg/kg (Rajeshwar et al., 2005).

Anti-oxidant activity

- Ethanolic extract of *Mucuna pruriens* showed in-vitro anti-oxidant effect (Sonpetkar et al., 2012).
- The ethanolic extract has also shown antilipid peroxidation property through the removal of super oxides and hydroxyl radicals (Tripathi and Upadhyay, 2001; Kumar DS and Muthu AK, 2010).

Anti-hyperlipidemic effect

The ethanolic extract of *Mucuna pruriens* has shown anti-hyperlipidemic effect in alloxan induces diabetic rats (Eze et al., 2012).

Anti-snake venom effect

Mucuna pruriens seed extract has shown protective effect against i.v. injection of Maja sputatrix (Malayan cobra) venom poisoning in rats (Fung et al., 2009; Tan et al., 2009).

Anti-bacterial activity

The methonolic ectract of *Mucuna pruriens* seed showed anti-bacterial activity aginst all strains like *Staphylococcus aureus, Bacillus pumillus, Escherichia coli* and *Vibrae cholera* (Rajeshwar et al., 2005).

Miscellaneous effect

- The aqueous extract showed protective effect against gentamicin induced nephrotoxicity and oxidative stress on rats (Modi et al., 2008).
- *Mucuna pruriens* has shown inhibitory effect on chlorpromazine-induced hyperprolactinaemia in man (Dhanasekaran S et al., 1978).
- Analgesic and anti-pyretic effect was also found with *Mucuna pruriens* (Lauk I et al., 1993).

2.4 The Plant: *Vicia faba*

FIGURE 2.4 *Vicia faba* seeds and pods

2.4.1 General profile

- Plant Name: *Vicia faba*
- Family: Leguminosae
- Geographical distribution: It is widely cultivated worldwide (Singh AK and Bhatt BP, 2013).
- Common Names: ‘‘broad bean’’ or ‘‘faba bean”, Bakala in Hindi (Kirtikar KR et al., 1996)

2.4.2 Macroscopic description

2.14 Macroscopic description of *Vicia faba* plant

Part(s)	Description
Leaves	• 10-25 cm long, pinnate and of a distinct grey-green color with 1-2.5 cm long flowers of five petals
Fruit	• Broad, leathery pod, green maturing to blackish-brown.

2.4.3 Phyyoconstituents

- L-DOPA (0.7 %) (Guggenheim M, 1913; Ingle PK, 2003).
- Carbidopa (Mohseni M and Golshani B, 2013).
- Condensed tannins, vicine, divicine, vicineane and convicine, crude protein (30.57%), crude fat (3.22%), crude fibre (2.73%), ash (3.61%) and carbohydrate (59.87%) (MdGulam et al., 2009).
- Excellent source of folates.
- Good amounts of vitamin B-6 (pyridoxine), thiamine (vitamin B-1), riboflavin and niacin.
- Fine source of minerals like iron, copper, manganese, calcium, magnesium. At 1062 mg or 23% of daily recommended levels, fava are one of the highest plant sources of potassium (USDA Nutrient Database, 2013).

2.4.4 Pharmacological studies

Anti-Parkinson's effect

- Parkinson's disease patients showed a significant improvement in their motor features after eating *Vicia faba* which was similar to the improvement measured after receiving 125 mg of levodopa plus 12.5 mg of carbidopa (Rabey et al., 1992).
- L-DOPA obtained from *V. faba* seed prolongs "On" period in patients with Parkinson's disease who have 'on-off' effect fluctuation (Apaydin et al., 2000).

Anticonvulsant effect

The *Vicia faba* seed extract have shown anticonvulsant effect (Mustafa and Mustafa, 2008).

Anti-oxidant effect

Vicine and *divicine* glycosides extracted from mature seeds show anti-oxidant effect (Yadava et al., 2013).

Analgesic and anti-inflammatory activity

- *Vicine* and *divicine* glycosides extracted from mature seeds show anti-inflammatory activity (Mohammed, 2012).
- The methanolic extract of leaves of *Vicia faba* has shown analgesic effect against acetic acid induced writhing method in mice (Rajesh and Sunil, 2013)

Muscle relaxant effect

Methanolic extract of seeds of *Vicia faba* has shown muscle relaxant activity at 600 mg/kg dose (Rajesh, 2014).

Anti-diabetic effect

Vicine and *divicine* glycosides extracted from mature seeds show anti-diabetic activity against streptozotocine-induced diabetes in rats (Hussein et al., 2014).

Anti-obesity effect

Cooked and germinated bean has shown weight lowering effect in rats (Al-Masri, 2015)

2.5 The Plant: *Bauhinia purpurea*

FIGURE 2.5 *Bauhinia purpurea* seeds and pods

2.5.1 General profile

- Plant Name: *Bauhinia purpurea*
- Family: Leguminosae
- Distribution: Throughout India, ascending to an altitude of 1,300 m in sub-Himalayan tract.
- Common Names: ‘‘kanchnar’’, orchid tree, butterfly tree (Kirtikar et al., 1996)

2.5.2 Macroscopical description

2.15 Macroscopic description of *Bauhinia purpurea* plant

Part(s)	Description
Bark	• Pale grey brown, fairly smooth to slightly fissured and scaly
Twigs	• Slender, light green slightly hairy, and angled, becoming brownish grey
Leaves	• Simple, alternate, base rounded to shallow-cordate, upto 12 cm, deeply 2-lobed at apex up to 1/3-1/2, 7-12 cm long, and equally wide, margin entire and the surfaces smooth and glabrous, and 9-

	or 11-nerved at base, the apex lobes rounded or obtuse to subacute, minute stipules 1-2 mm long, petioles puberulous to glabrous, 2.5-3.5 cm long leaf blades 4.5-11 cm long (Orwa C et al., 2009).
Fruit	• Brown, strap-shaped, not septate, elongated dehiscent pods • 15-30 cm long, upto 1.5-2.5 cm wide • Containing 10-15 shiny-brown, glabrous, dehiscent, rounded seeds • Fruit maturing in spring and summer • .Fruit does not attract wildlife (Orwa C et al., 2009).
Seeds	• Orbicular • 13-16 mm in diameter • 1-2 mm thick (Orwa C et al., 2009).
Flowers	• 6-10 flowered • Hypanthium, turbinate, purple to nearly white or at least purple-marked • Flower buds clavate (club-shaped), velvety, 3-4 cm long prior to anthesis • Fertile stamens 3 or 4, the anthers 6 mm long, versatile • Ovary superior; corolla of 5 narrow petals and constricted at base, oblanceolate, 3-5 cm long, claws 5-10 mm long, the banner purple- striate, 7 mm wide; • Calyx tubular, erupted by corolla along one side when flower fully expanding; calyx split into 2 valves with 5 teeth. In fall, before the leaves drop, Orchid-Tree is festooned with many showy and delightfully fragrant, five-inch-wide blossoms, the narrow purple, pink, and lavender petals arranged to closely resemble an orchid. • The flowers are followed by 12-inch-long, slender, brown, flat seed pods which usually persist on the tree throughout the winter (Orwa C et al., 2009).

5.3 Products

2.16 Products obtained from *Bauhinia purpurea* plant

Part(s)	Description
Food	• Leaves and flowers of various Bauhinia species are eaten as a side dish with rice, or used to flavour meat and fish. • Sometimes the seeds are edible.
Fodder	• *B.purpurea* was found to increase milk production in lactating buffaloes • Leaves make good fodder and are greedily eaten by sheep, goats and cattle with protein content estimated at 12.6%.
Fibre	• The bark of bauhinia is used to make rope and stems of smaller lianescent species are used for binding. • Used for binding
Tannin or dyestuff	• Used in leather industry in India.
Medicine	• Used to reduce swelling and bruises, and to ripen ulcerations and boils. • Decoctions of various plant parts are taken internally as a febrifugal, antidiarrhoeal and antidysenteric remedy and also it is used as an astringent. • In India, the bark is extensively applied in glandular diseases and as a poison antidote while the leaves are administered as cough medicine. • Flowers are laxative and used in curries and pickles.
Lipid	• High amounts of linolenic and oleic fatty acids (15% of anon-dryingoil) and low amounts of myristic and linolenic fatty acidsare presents in seeds.
Gumorresin	• The tree yield edible gum.
Fuel	• Used as fuel wood; its calorific valueis 4800 kcal/kg.

2.5.4 Phytoconstituents

- L-DOPA (2.2 %) (Ingle PK, 2003, Vellingiri et al., 2012).
- Glycosides
- Flavonoids
- Saponins
- Triterpenoids
- Phenolic compounds
- Oxepins
- Fatty acids and phytosterols (Kumar and Chandrashekhar, 2013).

2.5.5 Pharmacological studies

Anti-Parkinson's effect

The Ethanolic extract of *Bauhinia purpurea* seed at 200 mg/kg and 400mg/kg has shown a significant protection against haloperidol induced Parkinson's disease (Shaik et al., 2014).

Anti-hyperlipidemic and anti-obesity effect

- Methanolic extract of *Bauhinia purpurea* bark has shown anti-hyperlipidemic and anti-obesity efficacy in HFD induced male Wistar albino obese rats (Mopuri et al., 2010).
- Ethanolic extract of *Bauhinia purpurea* bark at dose 300 mg/kg showed anti-obesity effect (Padmaja et al., 2014).

Wound healing effect

Chloroform and methanolic extract of leaves of *Bauhinia purpurea* shows wound healing property (Ananth et al., 2010).

Antimalarial, anti-mycobacterial, antifungal and cytotoxic activities

Root extract exhibited antimalarial, antimycobacterial, antifungal and cytotoxic activities (Boonphong et al., 2007).

Anti-diarrheal activity

Ethanolic extract of leaves of *Bauhinia purpurea* shows anti-diarrheal activity (Mukharji et al., 1998).

Hypoglycaemic activity

Ethanolic extract of *Bauhinia purpurea* stems shows hypoglycaemic activity (Muralikrishna et al., 2008).

Antioxidant activity

Ethanolic extract of leaves of *Bauhinia purpurea* exhibited antioxidant activity (Silva et al., 2005).

Miscellaneous activity

- Ethanolic extract of *Bauhinia purpurea* stems shows cardiac activity (Muralikrishna et al., 2008).
- The aqueous alcoholic bark extract and aqueous root extract of *Bauhinia purpurea* stimulate the thyroid function in female mice (Panda et al., 2005).
- Aqueous extract of *Bauhinia purpurea* possesses good antinociceptive, anti-inflammatory, analgesic and anti-pyretic properties (Chandrashekhar et al., 2009a).
- Ethanolic extract of leaves of *Bauhinia purpurea* shows protective effect against gentamicin induced nephrotoxicity (Lakshmi et al, 2009).

CHAPTER 3

Materials & Methods

3.1 Experimental animals

Male SD rats weighing 250-300 g were used in the study. The animals were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22±2°C), humidity (55±5%) and light-dark cycles (12:12h). They were maintained under standard environmental conditions and were fed a standard rat chow diet with water given ad libitum. The study was approved by Institutional Animal Ethics Committee (IAEC), Parul institute of pharmacy, Parul University, Vadodara, Gujarat, India (PIPH 19/12, PIPH 12/14, PIPH 01/15).

3.2 Collection and authentication of plant material

Seeds of *Mucuna pruriens* (L.) DC. and *Vicia faba* were purchased from the authorised dealer whereas seeds of *Bauhinia purpurea* were collected from botanical garden of Parul University in months of summer. All three seeds were authenticated by Prof. Vinay Raole, Botany department of MS University, Vadodara, India.

3.3 Preparation of extracts

For the extraction, freshly collected seeds were dried in shade and pulverized to get a coarse powder. One kg seed powder of each of the plants was initially defatted with 750 ml of petroleum ether and then aqueous extracts were prepared by cold maceration method. After 24 h, the extracts were filtered using Whatman filter paper (No. 1) and

then concentrated under reduced pressure (bath temp. 50 °C) and finally dried in a vacuum desiccator (Modi et al., 2008).

3.4 Experimental Design

3.4.1 Induction of obesity

Male SD rats were acclimatized for 1 week. Obesity was induced in rats of all groups except normal control group by administration of HFD for 12 weeks. The composition of HFD was finalized after necessary modification in the reported study on obesity. The composition of HFD is mentioned in table 3.1 (Barnard RJ, 1993).

Table 3.1: Composition of HFD

Ingredients	Weight (g)	Energy (Kcal)
Powdered NPD	50	0.21
Animal fat	20	0.18
Sucrose	29.8	0.11
Sodium chloride	0.2	-
	100 g	0.5 Kcal/100 g

Preparation of HFD pellet

The HFD pellets were prepared every day throughout the study to prevent fungus contamination. Initially powdered NPD, sucrose and sodium chloride were mixed accordingly as given in table 3.1. Into this mixture melted animal fat was added and mixed until it became homogenous in a dough-like consistency. Then pellets were prepared as per the requirements.

3.4.2 Grouping of animals

The rats were divided into nine groups consisting of six rats in each as follows:

Group I: Normal protein diet (NPD)

Group II: HFD

Group III: HFD + L-DOPA (12.5 mg/kg, p. o.)

Group IV: HFD + AEMP (200 mg/kg, p. o.)

Group V: HFD + AEMP (400 mg/kg, p. o.)

Group VI: HFD + AEVF (300 mg/kg, p. o.)

Group VII: HFD + AEVF (600 mg/kg, p. o.)

Group VIII: HFD + AEBP (300 mg/kg, p. o.)

Group IX: HFD + AEBP (600 mg/kg, p. o.)

Group I was fed NPD, while groups II to IX were fed HFD for 12 weeks, that is throughout the study. At the end of 8 weeks, groups II to IX were treated with test extract or standard drug once daily for 4 weeks along with HFD.

3.4.3 Measurement of anti-obesity parameters

Body weight and food intake were measured every week while BMI and serum TC, TG and HDL levels were measured at the end of 4, 8 and 12 weeks. The epididymal WAT mass and brain dopamine levels were measured at the end of 12 weeks.

3.4.3.1 Body weight measurement

Body weight of each rat was measured every week till 12 weeks by calibrated weighing machine (weighing machine was same throughout the study to minimize the error).

3.4.3.2 Food intake

The food intake was calculated per group every day throughout the study every morning at 10:00 am. The remaining feed material was discarded and every day fresh food was placed.

3.4.3.3 BMI determination

The body weight and body length (nose to anus length) were used to determine the BMI (Bernardis, 1970). The measurements were made in anaesthetized rats (anaesthetized by 0.1 ml intra peritoneal administration of 1% sodium barbiturate). The BMI was calculated using (3.1)

$$\text{BMI} = \text{body weight (g)}/\text{length}^2\ (\text{cm}^2) \quad \textbf{Equation 3.1}$$

3.4.3.4 Collection of blood samples and estimation of TC, TG and HDL levels

At the end of fourth, eighth and twelfth weeks, blood was collected under inhalation anaesthesia by retro-orbital puncture from overnight fasted animals. Blood was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 4000-5000 rpm for 15 min and analysed for serum TC, TG and HDL levels. TC, TG and HDL were estimated by using a Bayer diagnostic kit (Bayer Diagnostic India Ltd.).

Assay for serum TC: The serum level TC was quantified by method described by Stein (1987). 1000 µl of the reagent was added to each of the sample and standard. This was incubated at 20-25 ºC for 10 min after mixing and absorbance of the sample (A_{sample}) and standard (A_{std}) was measured against the reagent blank within 30 min at 546 nm. The concentration of sample was calculated using (3.2).

$$TC = A_{sample} / A_{std} * 196.86 \text{ mg/dL} \quad \textbf{Equation 3.2}$$

Where, A_{sample} is the Absorbance of sample

A_{std} is the Absorbance of std

Assay for serum TG: The serum TG level was analysed after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 1000 µl of the reagent was added to each of the sample and standard. This was incubated at 20-25 ºC for 10 min after mixing and absorbance of the sample (A_{sample}) and stdandard (A_{std}) was measured against the reagent blank within 30 min at 546 nm using. The concentration of sample was calculated using (3.3).

$$TG = A_{sample} / A_{std} * 194.00 \text{ mg/dL} \quad \textbf{Equation 3.3}$$

Where, A_{sample} is the Absorbance of sample

A_{std} is the Absorbance of std

Assay for serum HDL: The serum HDL level was determined by method of Wacnic and Alber (1978). 1000 µl of the reagent was added to each of the sample and standard. LDL and VLDL and chylomicron fractions were precipitated by addition of phosphotungustic acid in the presence of magnesium ions. This mixture was allowed to stand for 10 min at room temperature and centrifuged for 10 min at 4000 rpm. The supernatant represented the HDL-C fraction and was determined.

3.4.3.5 Fat pad analysis

At the end of the 12 weeks, animals were decapitated between 09:00 and 12:00 h. After sacrificing by decapitation, the epididymal WAT was dissected out. The collected fat was weighed immediately and compared with the other groups (Loncar et al., 1988).

3.4.3.6 Brain dopamine levels

Preparation of tissue extract: On the last day of experiment, rats were sacrificed and whole brain was dissected out, weighed and was homogenized in 3 ml HCl butanol in a cool environment. The sample was subsequently centrifuged for 10 min at 2000 rpm. 0.8 ml of the supernatant phase was removed and added to an eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M HCl. After 10 min, the tube was shaken and centrifuged under the same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay

0.02 ml of the HCL phase+0.005 ml 0.4 M HCL+0.01 ml EDTA

↓

0.01 ml iodine solution was added for oxidation

↓

After 2 min, 1 ml sodium thiosulphate in 5 M sodium hydroxide was added to stop the reaction

↓

10 M acetic acid was added 1.5 min later

↓

Solution was then heated to 100 ^{o}C for 6 min

↓

Excitation and emission spectra were read (330 to 375 nm) in a spectrofluorophotometer (Shimadzu RF-5301 PC) when the samples again reached room temperature.

Tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) were compared with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank).Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005 ml bidistilled water and 0.1 ml HCl butanol to 20 ng of dopamine standard 1 (Jacobowitz and Richardson, 1978); Schlumfjf et al., 1974).

3.4.4 Phytochemical evaluation

3.4.4.1 Preliminary phytochemical screening: All seeds extracts were subjected to preliminary phytochemical tests for the identification of chemical constituents like carbohydrates, glycosides, amino acids, flavonoids, fixed oils, tannins, gum mucilage using standard methodology.

3.4.4.2 HPTLC fingerprinting of all extracts

Preparation of std L-DOPA solution: A stock solution of L-DOPA (200 µg/ml) was prepared by dissolving an accurately weighed 2 mg L-DOPA standard in 2 ml of

anhydrous formic acid and volume was made up to 10 ml with methanol in a volumetric flask. Standard working solution of 2 µg/ml concentration was prepared by diluting 0.1 ml stock solution up to 10 ml with methanol. Ten microliters from prepared standard solution was spotted on the TLC plate to obtain final concentration of 20 ng/spot.

Preparation of all three plant extract solution: An accurately weighed 1000 mg of dry aqueous extract was transferred in to 10 ml volumetric flask and 5 ml of anhydrous formic acid was added, sonicated for 10 min and volume was made upto 10 ml with methanol. The extract was filtered on Whatman filter paper (No. 1). Ten microliters of above solution was spotted on the TLC plate.

Qualitative L-DOPA determination: The Mobile phase *n*-butanol: acetic acid: water (4:1:1, v/v/v) was used for present study. Chromatography was performed on Merck Silica gel 60F254 TLC precoated aluminium plates. 10µl of freshly prepared samples were applied on the plate as a band of 10 mm width with the help of LINOMAT VR Automatic sample spotter at the distance of 10 mm from edge of the plate. The plate was developed to a distance 75mm in a CAMAG twin trough chamber (10*10 cm) previously equilibrated with mobile phase for 20 minutes. After development, densitometry evaluation of plate was performed at 254 nm in absorption mode using TLC scanner 3 linked to WinCats Software (CAMAG).

Quantification of L-DOPA in extracts: The concentration of L-DOPA in all plant extracts were determined by (3.4).

$$Au/As=Cu/Cs \quad \textbf{Equation 3.4}$$

Where , Au= AUC of test

As= AUC of std L-DOPA

Cu= concentration of test

Cs= concentration of std L-DOPA

3.4.5 Statistical Analysis

All data were presented as mean±standard error of means (SEM). One-way analysis of variance (ANOVA) followed by Tukey's test was used for statistical analysis to compare more than two groups, while two way ANOVA followed by Bonferroni test was used to compare values of different time period of the same group. P values of less than 0.05, 0.01 and 0.001 were considered as significant.

CHAPTER 4

Results

4.1 Effect of aqueous extract of *M. pruriens* seeds

4.1.1 Body weight

Body weight was measured every week till twelve weeks. The body weights of all HFD treated groups (II, III, IV & V) were significantly increased compared to the control group (group I) for first 8 weeks. Treatment with L-DOPA and AEMP (200 and 400 mg/kg) was given from 8 to 12 weeks in groups III, IV and V respectively. After 12 weeks, the L-DOPA and AEMP (400 mg/kg) groups had significantly lower ($p<0.001$) mean body weights as compared to only HFD group (Group II). The mean body weight in the HFD group increased by 46.67 g after 12 weeks of experimental period ($p<0.01$), whereas L-DOPA and AEMP (400 mg/kg) group lost 23.33 g and 30.00 g respectively. In normal and AEMP (200 mg/kg) treated groups mean body weight gain was found to be 13.33 g and 6.67 g respectively (Table 4.1).

TABLE 4.1: Effect of AEMP on body weights (g) at different time interval

Treatment period	Normal	HFD	HFD + L-DOPA	HFD + AEMP (200 mg/kg)	HFD + AEMP (400 mg/kg)
Initial	321.67 ± 11.67	268.34 ± 14.24	270.00 ± 15.00	303.34 ± 3.33	303.34 ± 8.81
8 weeks	363.3 ± 15.275 (41.63)	420.00 ± 35.11 (152.3) *	390.00 ± 30.00 (120.67)*	460.00 ± 25.16 (147.3)*	443.33 ± 12.01 (140.0)*
12 weeks	376.7 ± 14.53 (13.33)	466.67 ± 38.44 (46.67)##	366.66 ± 33.33 (-23.33)###	466.67 ± 33.33 (6.67)##	413.33 ± 6.67 (-30.00)###

Values are expressed as mean ± SEM, n=6;

* g of body weight gained during the first 8 weeks of the experimental period;

##p<0.01, ###p<0.001 g of body weight altered during 8 to12 weeks experimental period (analysed by one way ANOVA followed by Tukey's test).

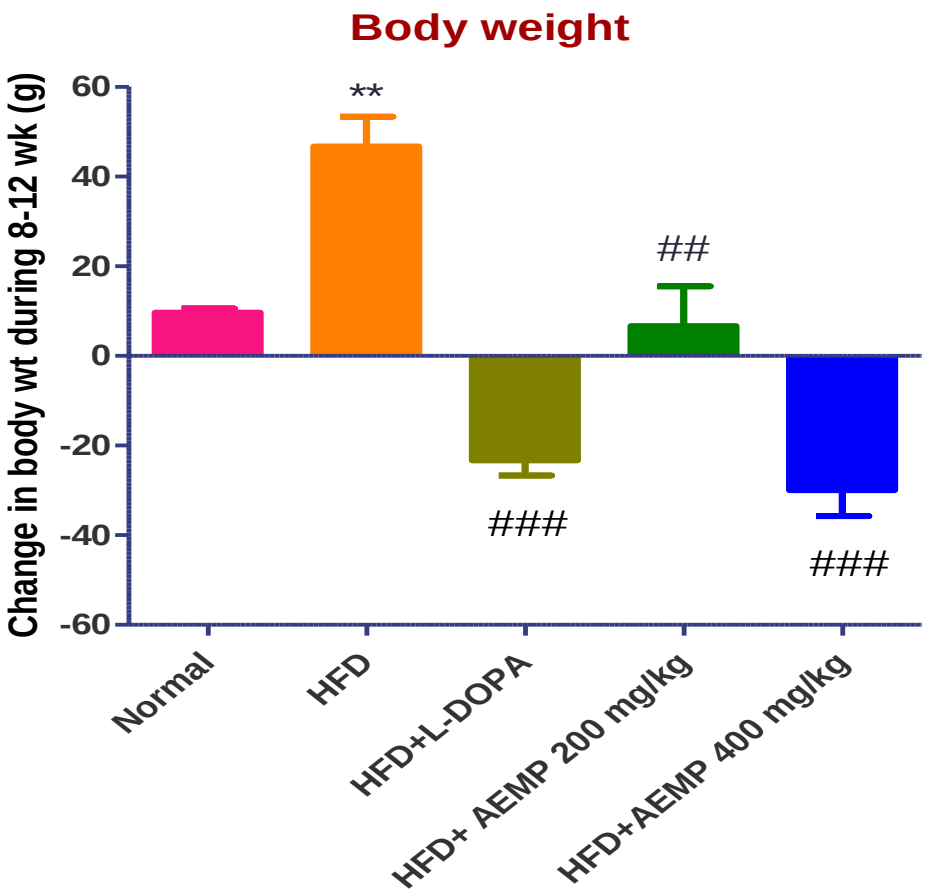


FIGURE 4.1: Effect of AEMP on body weight.

Each bar represents mean ± SEM values of body weight, n=6; **$p<0.01 compared with group I after 12 weeks administration of HFD; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared with group II after 12 weeks administration of HFD (analysed by one way ANOVA followed by Tukey's test).

4.1.2 Food intake

Feeding of HFD caused a significant increase in food intake per week among the HFD treated rats as compared to the normal diet-fed rats. The rats treated with AEMP (200 mg/kg, p.o. and 400 mg/kg, p.o.) showed a significant decrease in food intake as compared to the HFD group. Standard L-DOPA treated group also exhibited a significant ($p<0.001$) reduction in food intake as compared to the HFD group (Fig.4.2)

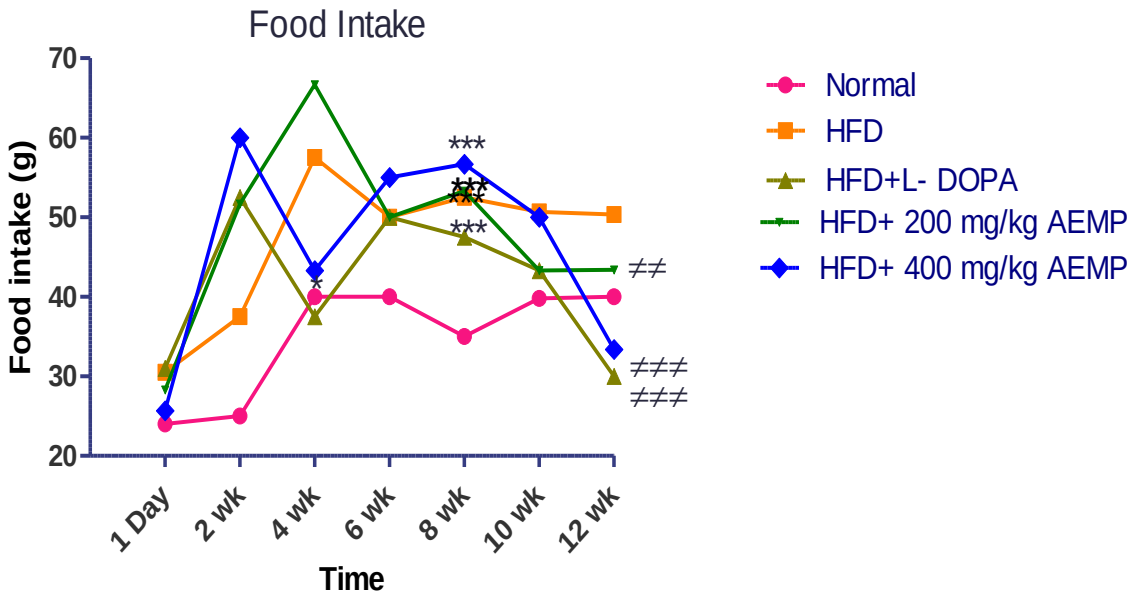


FIGURE 4.2: Effect of AEMP on food intake.

Each line represents values of average food intake for each group, n=6; ***$p<0.001$, compared to group I after 8 weeks; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to Group II after 12 weeks (analysed by two way ANOVA followed by Bonferroni test)

4.1.3 Body mass index

The BMI was measured for each animal every week till 12 weeks. Group II showed significantly ($p<0.01$) increased BMI (0.84± 0.05) as compared to Group I (0.60±0.005) after 12 weeks. The data showed significant ($p<0.01$) lower BMI in L-DOPA treated group (0.61± 0.051) as compared to HFD treated group after 12 week of induction, whereas no significant changes were observed in AEMP (200 mg/kg and 400 mg/kg) treated group as compared to Group II. AEMP treated groups showed preventive effect (Fig. 2).

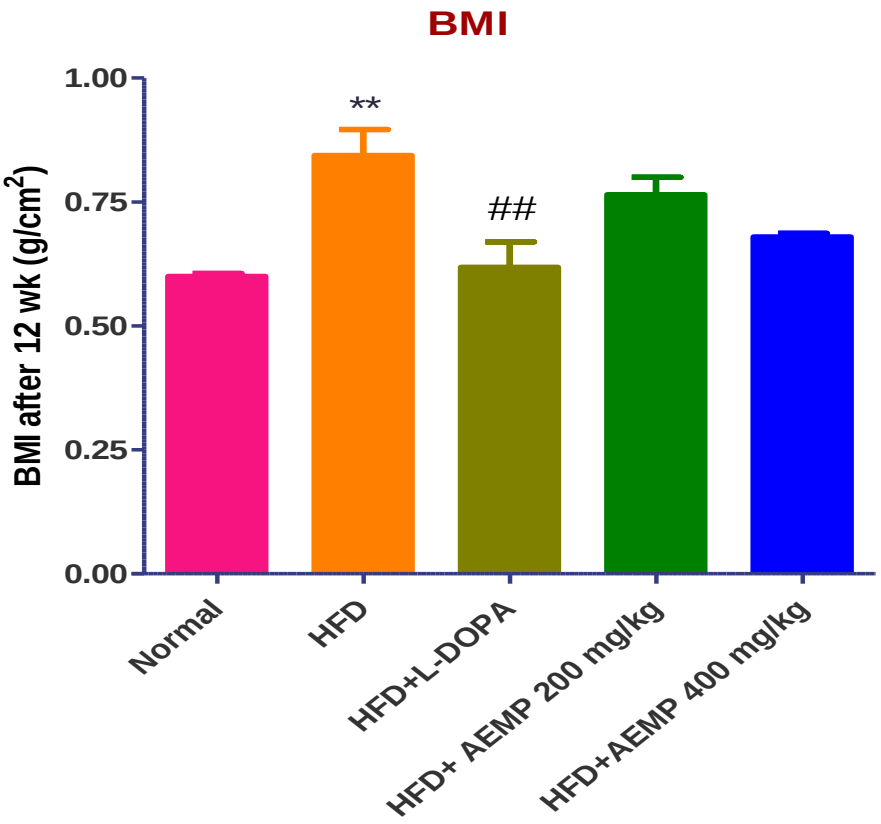


FIGURE 4.3: Effect of AEMP on BMI.

Each bar represents the mean ± SEM values of BMI, n=6; **$p<0.01$ compared with normal group after treatment; $^{##}p<0.01$ compared to Group II (analysed by one way ANOVA followed by Tukey's test).

4.1.4 White adipose tissue

Feeding a high-fat diet for 12 weeks produced a significant ($p<0.01$) increase in epididymal WAT weight (6.60±0.73) of HFD treated group as compared to normal diet fed rats (3.16±0.14). There was a significant ($p<0.01$) reduction in the epididymal fat mass in the L-DOPA (3.81±0.23) and AEMP (400 mg/kg) (4.38±0.32) treated groups, while no significant alteration was observed in AEMP (200 mg/kg) group as compared to HFD (Fig.4.4) treated group.

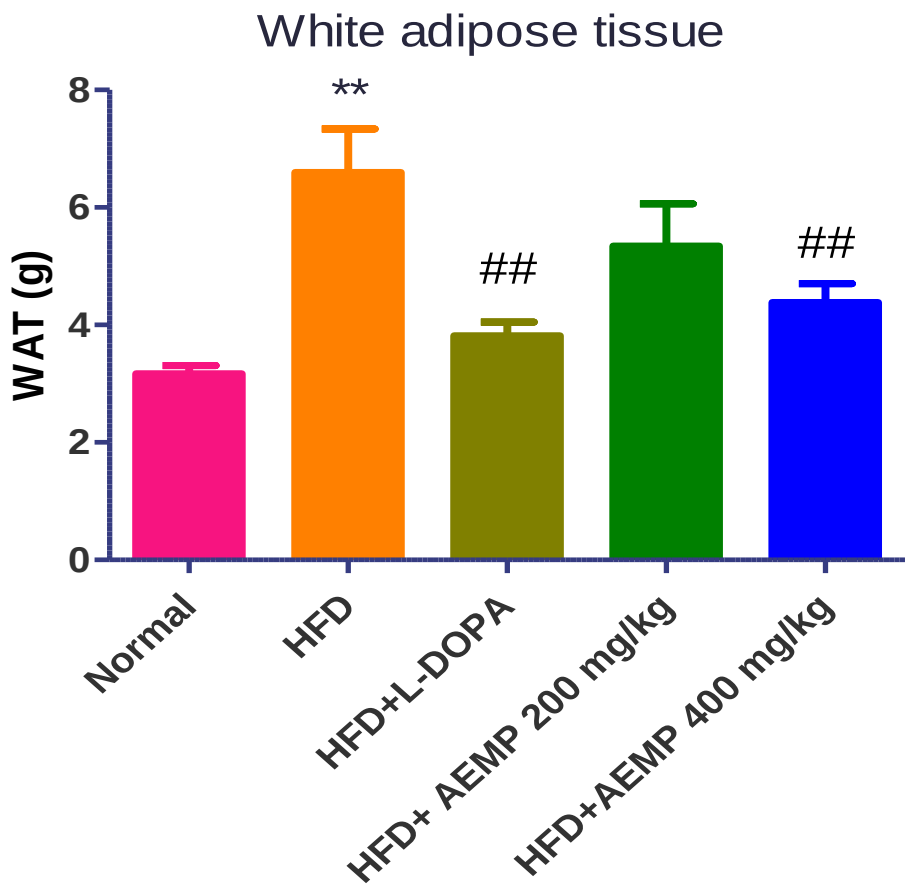


FIGURE 4.4: Effect of AEMP on white adipose tissue.

Each bar represents the mean ± SEM values of WAT weights, n=6; ******$p<0.01$ compared to group I, $^{\#\#}p<0.01$ compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.1.5 Serum lipid profile

As shown in Table 4.2, the L-DOPA and AEMP (200 mg/kg and 400 mg/kg) treated groups showed significantly lower serum TG levels (118.3±4.4, 95.33±4.84 and 94.33±21.96 respectively) than in HFD group (155.0±5.29). However, no alteration was found in TC and HDL levels by treatment with either drug.

TABLE 4.2: Effect of AEMP on serum levels of TG, TC and HDL (after the 12 weeks experimental period)

Serum level (mg/dl)	Normal	HFD	HFD + L-DOPA	HFD + AEMP (200 mg/kg)	HFD + AEMP (400 mg/kg)
TG	75.33±7.86	155.0±5.29**	118.3±4.41#	95.33±4.84#	94.33±21.96#
TC	58.67±10.37	70.67±6.489	85.67±3.33	63.00±9.07	67.33±2.02
HDL	37.93±1.67	40.33±4.48	48.70±3.2	37.47±5.23	42.23±1.39

Results are presented as means ± SEM, n=6; **p<0.01compared to normal group; #p<0.05compared to HFD group (analysed by one way ANOVA followed by Tukey's test).

4.1.6 Brain dopamine levels

Statistical analysis of brain dopamine levels showed significant ($p<0.05$) difference between the normal (0.86±0.02) and HFD (0.12±0.009) treated rats. L-DOPA (0.82±0.02) and AEMP (200 mg/kg and 400 mg/kg) treated groups (1.24±0.32 and 1.081±0.06) showed significant ($p<0.01$) increase in brain dopamine levels as compared to the HFD group (Fig. 4.5).

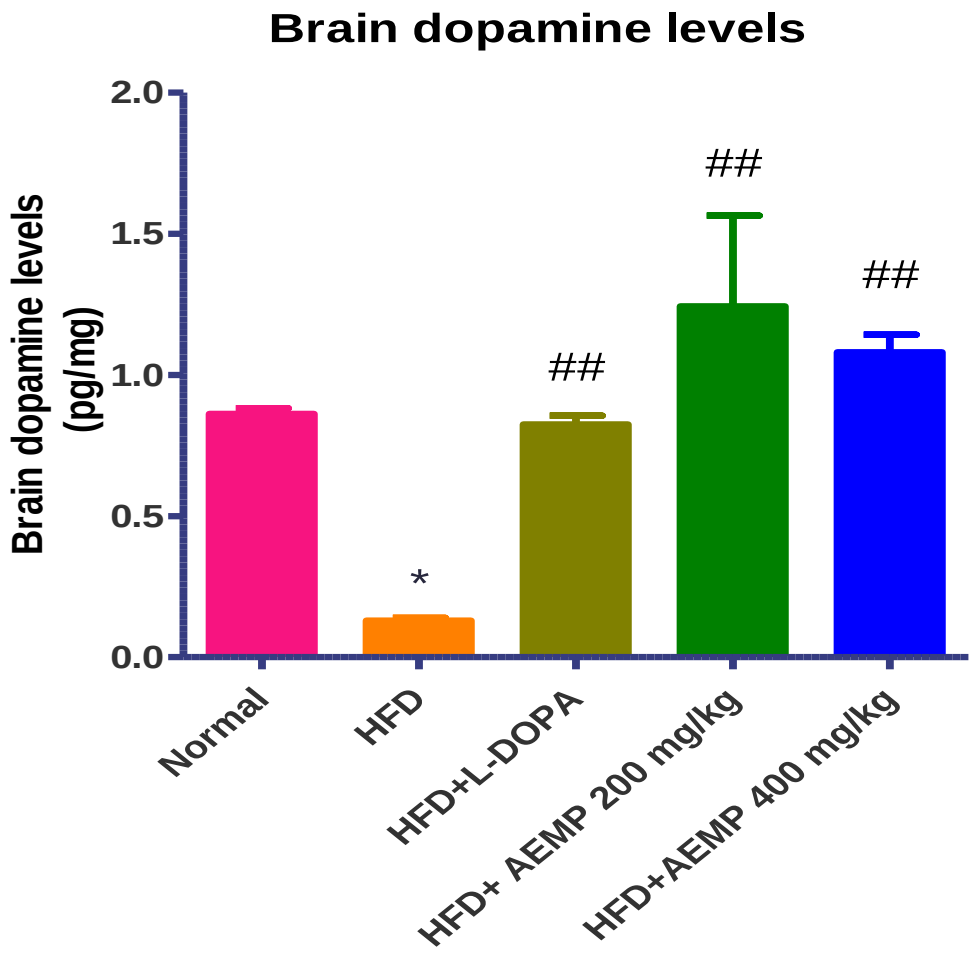


FIGURE 4.5: Effect of AEMP on brain dopamine level

Each bar represents the mean ± SEM of brain dopamine levels, n=6;*****$p<0.05$ compared to group I; $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared to Group II (analysed by one way ANOVA followed by Tukey's test).

4.1.7 Correlation between brain Dopamine level and body weight

The graphs showed the body weight loss in all the treated animals as compared to HFD group with corresponding increased brain dopamine levels.

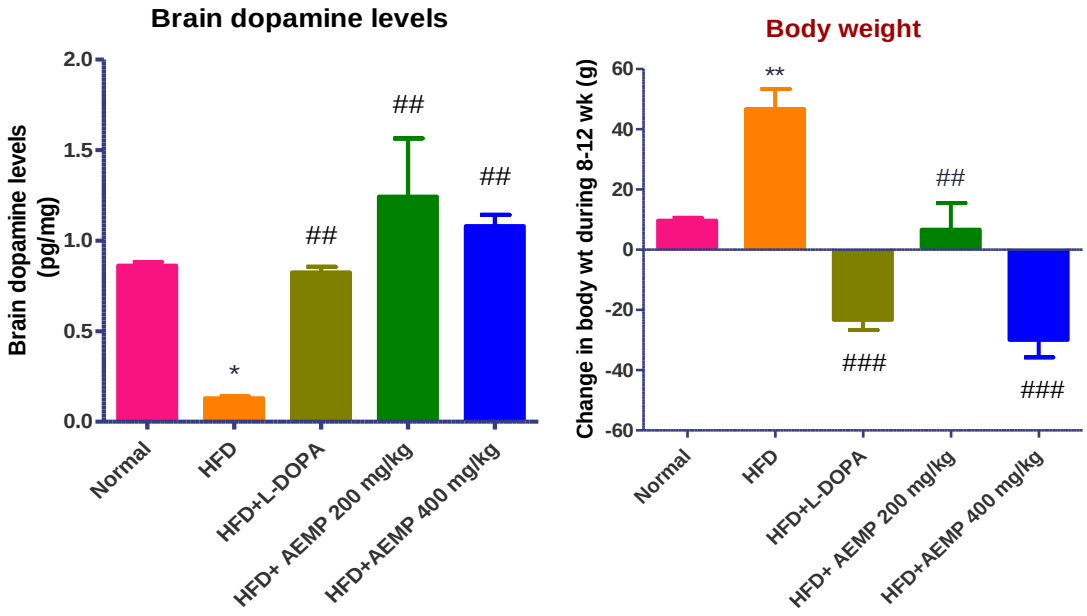


Figure 4.6 Brain dopamine and corresponding increased brain dopamine levels in AEMP treated group

Each bar represents the mean ± SEM, n=6; *****p<0.05, ******p<0.01 compared to group I; $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test).

4.2. Effect of aqueous extract of *V. faba* seeds

4.2.1 Body Weight

Body weight was measured every week till twelve weeks. The body weights of all groups (II, III, IV & V) were significantly increased compared to the control group (group I) for first 8 weeks. After 12 weeks, the L-DOPA and AEVF (600 mg/kg) groups had significantly ($p<0.05$) lower mean body weights than HFD group. The mean body weight in the HFD group increased significantly ($p<0.05$) by 46.67 g while L-DOPA and AEVF (600 mg/kg) group showed significant weight loss by 23.30 g and 13.03 g respectively between 8 to 12 weeks of experimental period. In normal and AEVF (300 mg/kg) treated groups mean body weight gain was found to be 13.33 g and 16.67 g respectively (Table 4.3, Fig. 4.6).

TABLE 4.3: Effect of AEVF on body weights (g) at different time intervals

Treatment Period	Normal	HFD	HFD + L-DOPA	HFD + AEVF (300 mg/kg)	HFD + AEVF (600 mg/kg)
Initial	321.67 ± 11.67	268.34 ± 14.24	270.00 ± 15.00	303.34 ± 3.33	303.34 ± 8.81
8 weeks	363.3 ± 15.275 (41.63)	420.0 ± 35.11 (152.3) *	390.0 ± 30.00 (120.67)*	510.0 ± 25.16 (147.3)*	453.33 ± 12.01 (140.0)*
12 weeks	376.7 ± 14.53 (13.33)	466.7 ± 38.44 (46.67)$^{\#}$	366.7 ± 33.33 (-23.3)$^{\#\#}$	526.7 ± 33.33 (16.67)	460.33 ± 6.67 (-13.03)$^{\#\#}$

Values are expressed as means ± SEM, n=6;

* g of body weight gained during the first 8 weeks of the experimental period.

$^{\#}p<0.05$, $^{\#\#}p<0.01$g of body weight altered during 8 to 12 weeks experimental period (analysed by one way ANOVA followed by Tukey's test).

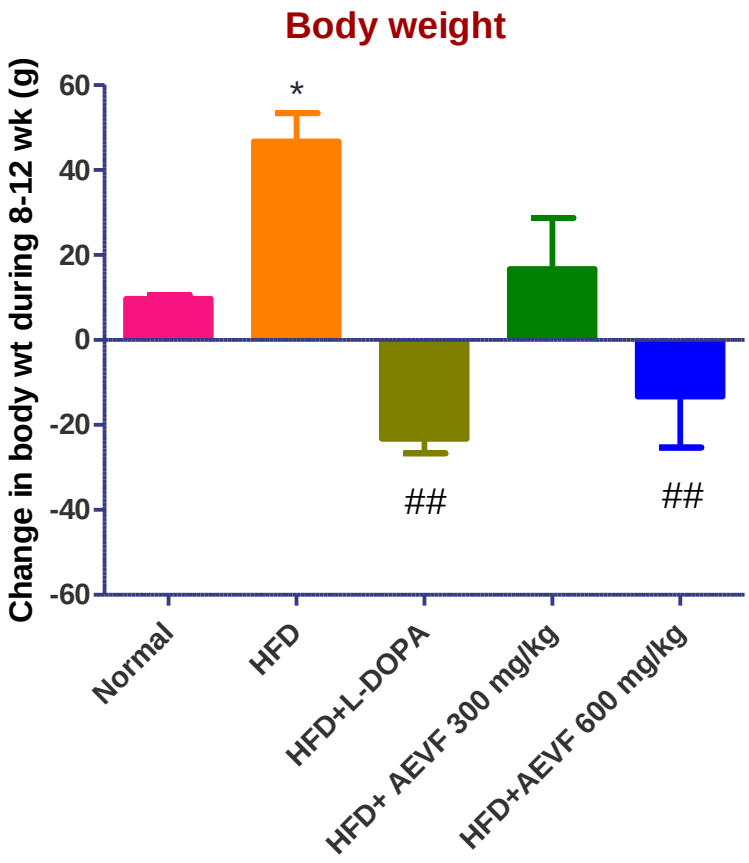


FIGURE 4.7: Effect of AEVF on body weight.

Each bar represents the mean ± SEM, n=6; *p<0.05 compared with group I after 12 weeks induction of HFD; ## p<0.01 compared with group II after 12 weeks (analysed by one way ANOVA followed Tukey's test).

4.2.2 Food intake

There was a significant increase in food intake per week among the HFD treated rats as compared to the normal diet-fed rats up to 8 weeks. The rats treated with AEVF (300 mg/kg, p.o. and 600 mg/kg, p.o.) showed a significant decrease in food intake as compared to the HFD group. L-DOPA treated group also exhibited a significant reduction in food intake as compared to the HFD group (Fig.4.7).

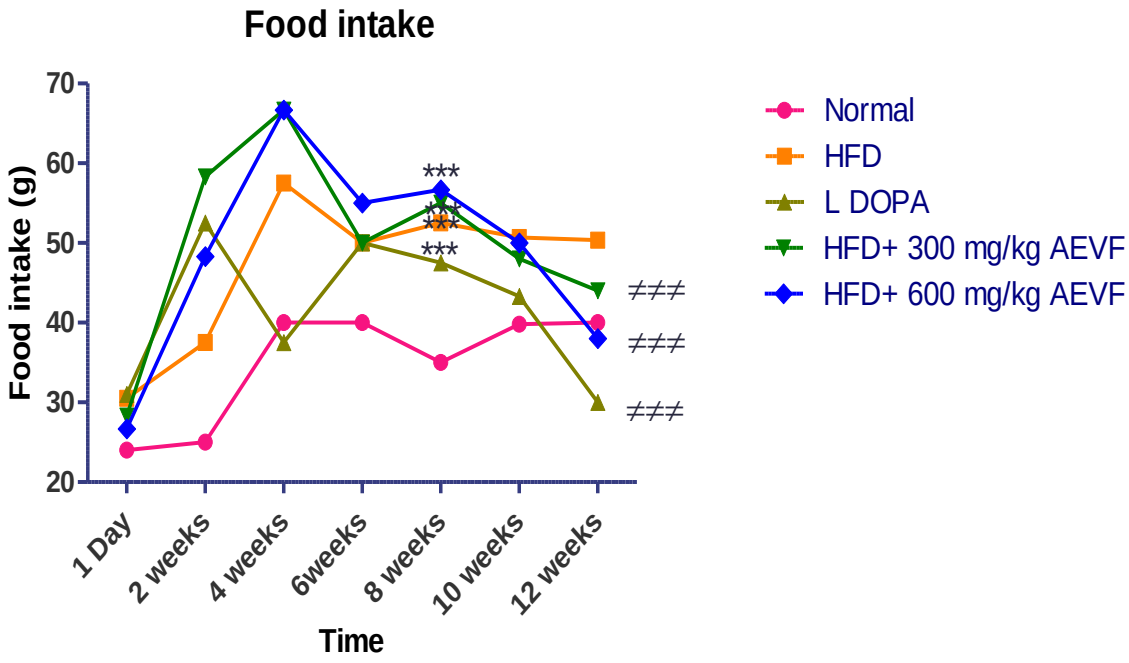


FIGURE 4.8: Effect of AEVF on food intake.

Each line represents values of average food intake for each group, n=6; ***p<0.001 compared to group I after 8 weeks; ###p<0.001 compared to Group II after 12 weeks (analysed by two way ANOVA followed by Bonferroni test)

4.2.3 Body mass index

HFD treated rats showed significant increase in BMI (0.84±0.05) as compared to control group (0.60±0.005) when measured every week till 12 weeks. L-DOPA treated group showed significant lower BMI whereas AEVF (300 mg/kg and 600 mg/kg) treated group showed no significant change in the BMI (0.77±0.04 and 0.67±0.05) as compared to HFD treated group. However, AEVF treated groups showed preventive effect (Fig.4.8).

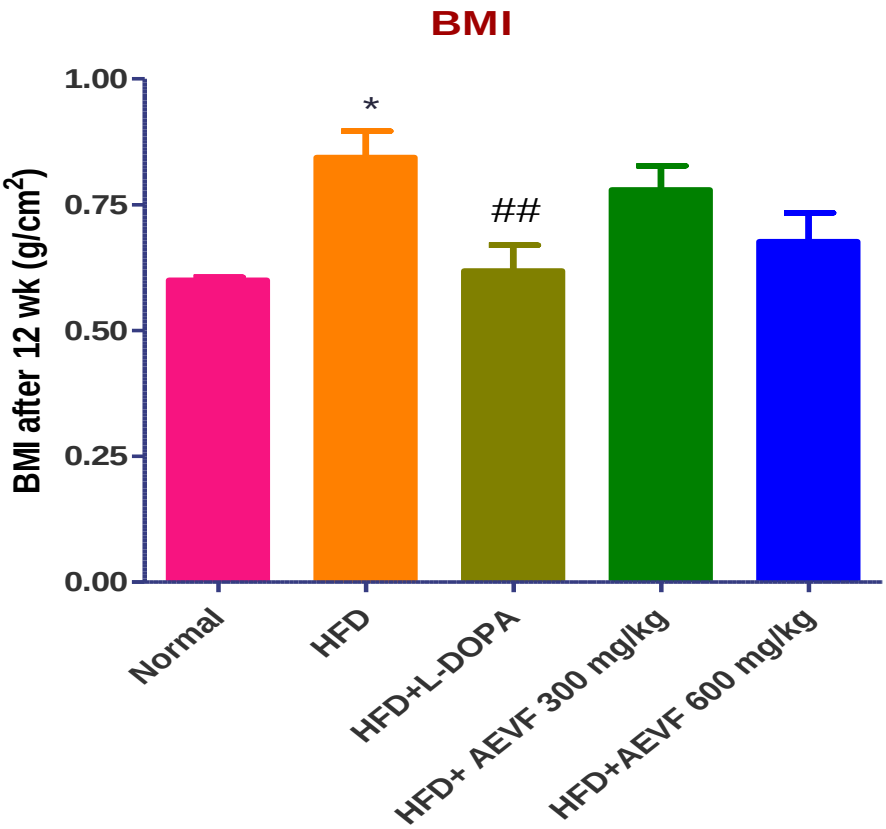


FIGURE 4.9: Effect of AEVF on BMI.

Each bar represents the mean ± SEM, n=6; *p<0.05 compared to group I, ##p<0.01 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.2.4 White adipose tissue

Feeding a high-fat diet for 12 weeks produced a significant (p<0.05) increase in epididymal WAT weight (6.26±0.48) of HFD treated group as compared to normal diet fed rats(3.30±0.05). There was a significant reduction in the epididymal fat mass in the L-DOPA (3.81±0.23) treated group while no significant alteration wera observed in AEVF (600 mg/kg and 300 mg/kg) groups as compared to HFD (Fig. 4.9).

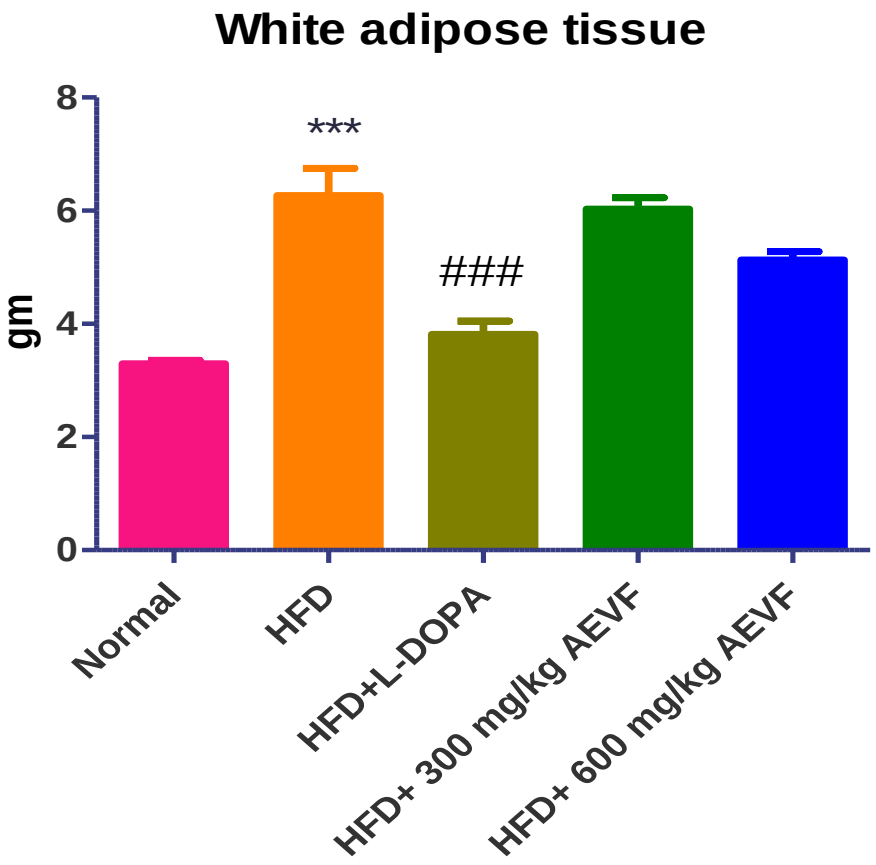


FIGURE 4.10: Effect of AEVF on white adipose tissue.

Each bar represents the mean ± SEM, n=6; ***p<0.001 compared to group I, ###p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.2.5 Serum lipid profile

Feeding of HFD caused a significant ($p<0.001$) increase in serum levels of TG as compared to normal diet fed rats. L-DOPA and AEVF (300 mg/kg and 600 mg/kg) treated groups showed significantly ($p<0.05$) lower serum TG levels than in HFD group. However, no alteration was found in TC and HDL levels by treatment with either drug (Table 4.4)**.**

TABLE 4.4: Effect of AEVF on serum level of TG, TC and HDL (after the 12 weeks experimental period)

Serum level (mg/dl)	Normal	HFD	HFD + L-DOPA	HFD + AEVF (300 mg/kg)	HFD + AEVF (600 mg/kg)
TG	75.33 ± 7.86	155.0 ± 5.29***	118.3 ± 4.41#	97.33 ± 4.84#	94.33 ± 21.96#
TC	58.67 ± 10.37	70.67 ± 6.48	76.67 ± 8.50	96.00 ± 4.93	67.33 ± 11.53
HDL	37.93 ± 1.67	40.33 ± 4.48	48.70 ± 3.2	44.70 ± 3.20	43.33 ± 2.18

Results are presented as means ± SEM, n=6; ***$p<0.001$compared to normal group; #$p<0.05$ compared to HFD group by one way ANOVA followed by Tukey's test.

4.2.6 Brain Dopamine levels

Brain dopamine levels of HFD group (0.12±0.006) was significantly (p<0.001) lower as compared to the control group (0.89±0.03). However, groups administered with L–DOPA and AEVF (300 mg/kg and 600 mg/kg) showed significant (p<0.001) increase in brain dopamine levels (0.59±0.02 and 0.67±0.004) as compared to the HFD group (Fig. 4.10).

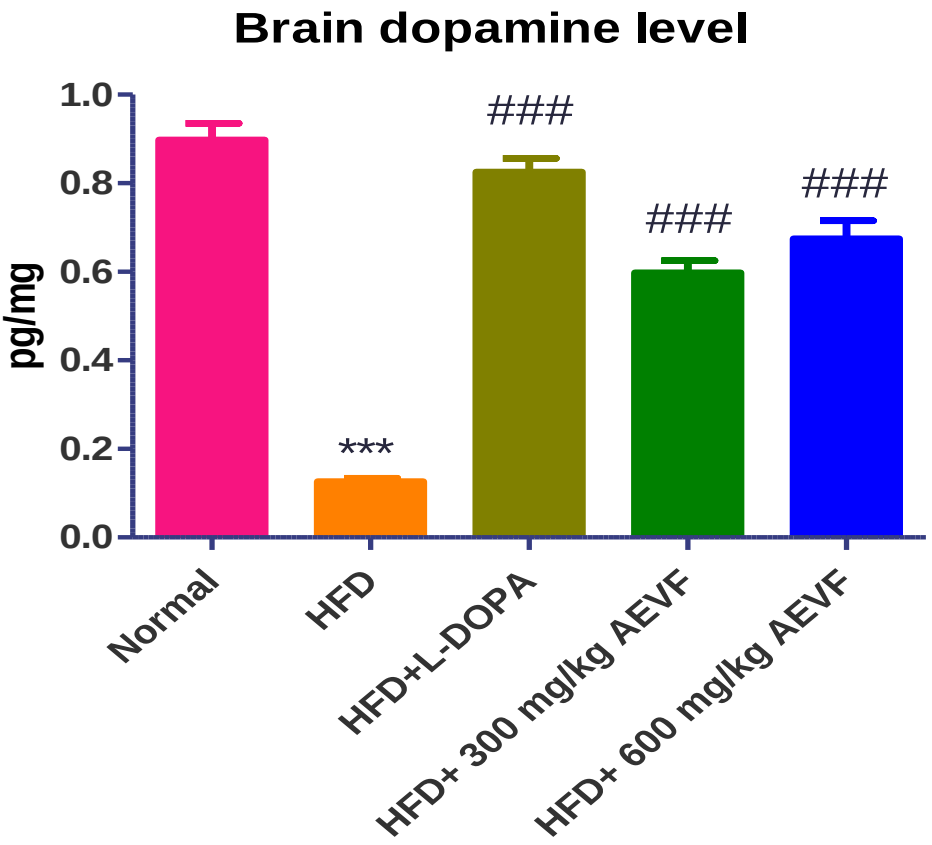


FIGURE 4.11: Effect of AEVF on brain dopamine level.

Each bar represents the mean ± SEM, n=6; ***p<0.001 compared to group I, ###p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.2.7 Correlation between brain Dopamine levels and body weight

The graphs showed the body weight loss in all the treated animals except AEVF 300 mg/kg as compared to HFD group with corresponding increased brain dopamine levels.

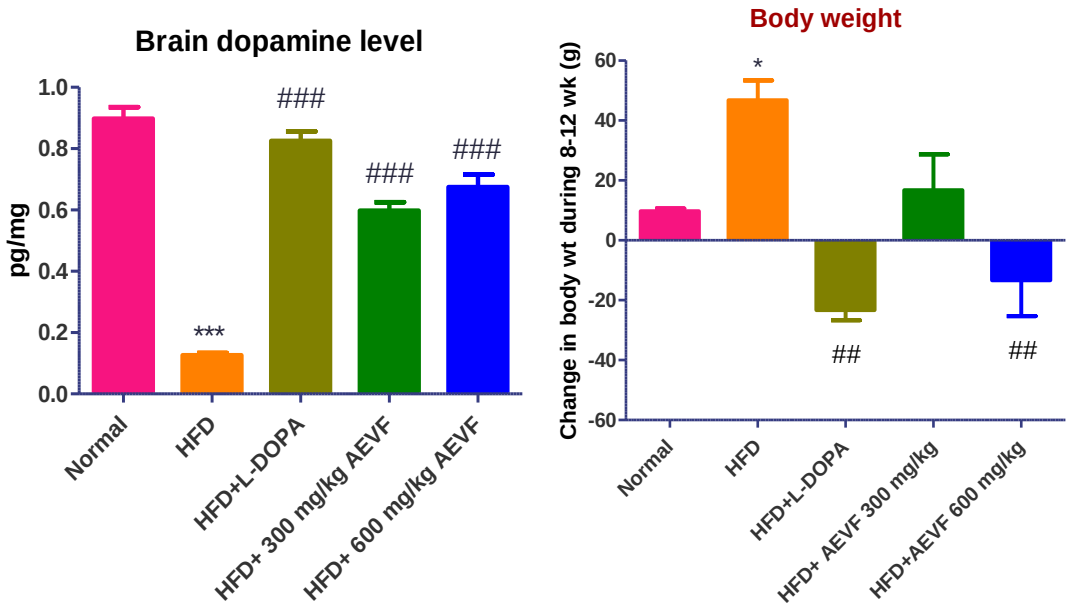


FIGURE 4.12 Brain dopamine and corresponding increased brain dopamine levels in AEVF treated group

Each bar represents the mean ± SEM, n=6; *p<0.0 =5, ***p<0.001 compared to group I, ##p<0.01, ###p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.3 Effect of aqueous extract of *B. purpurea* seeds

4.3.1 Body weight

Body weight was measured every week till twelve weeks. The body weights of all groups (II, III, IV & V) were significantly increased compared to the control group (group I) for first 8 weeks. After 12 weeks the L-DOPA and AEBP (300 mg/kg and 600 mg/kg) groups had significantly ($p<0.05$) lower mean body weights than HFD group. The mean body weight in the HFD group increased by 46.67 g after 12 weeks of experimental period, whereas L-DOPA and AEBP (300 mg/kg and 600 mg/kg) group lost 23.3 g , 6.67 and 30.00 g respectively (Table 4.5, Fig. 4.11).

Table 4.5: Effect of AEBP on body weights (g) at different time interval

Treatment Period	Normal	HFD	HFD + L-DOPA	HFD + AEBP (300 mg/kg)	HFD + AEBP (600 mg/kg)
Initial	321.67 ± 11.67	268.34 ± 14.24	270.00 ± 15.00	298.30 ± 11.67	255.00 ± 17.55
8 weeks	363.3 ± 15.275 (41.63)	420.0 ± 35.11 (152.3) *	390.0 ± 30.00 (120.67)*	490.0 ± 5.00 (191.7)*	410.00 ± 32.14 (155.0)*
12 weeks	376.7 ± 14.53 (13.33)	466.7 ± 38.44 (46.67)$^{\#}$	366.7 ± 33.33 (-23.3)$^{\#\#\#}$	483.34 ± 18.55 (-6.67)$^{\#\#}$	380.00 ± 32.14 (-30.00)$^{\#\#\#}$

Values are expressed as mean ± SEM, n=6

* g of body weight gained during the first 8 weeks of the experimental period.

$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ g of body weight altered during 8 to 12 weeks experimental period (analysed by one way ANOVA followed by Tukey's test).

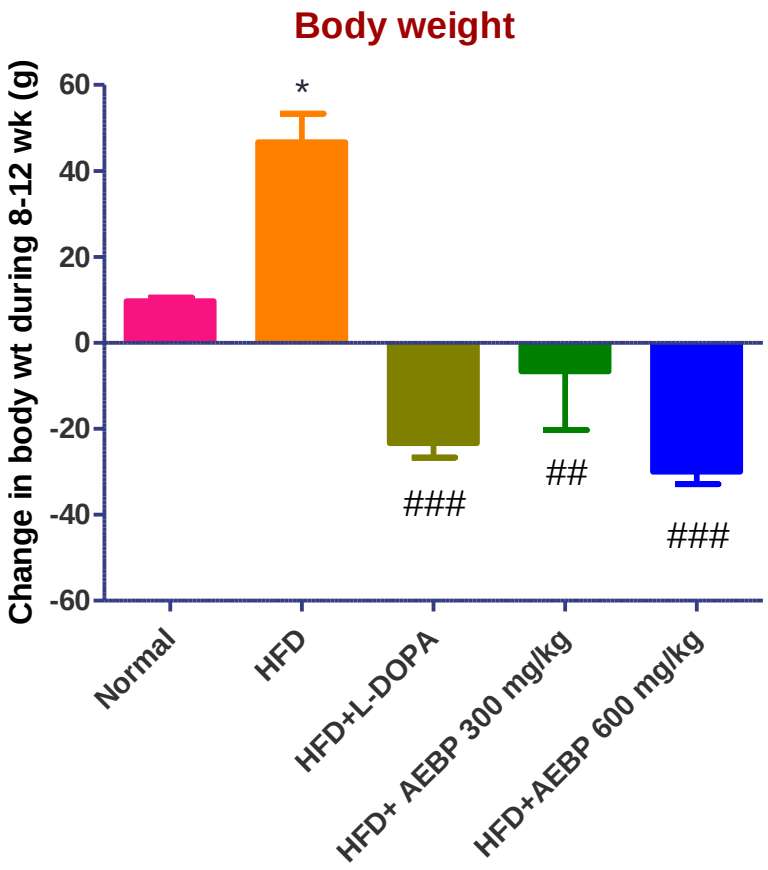


FIGURE 4.13: **Effect of AEBP on body weight**

Each bar represents the mean ± SEM, n=6;* $p<0.05$ compared to group I; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to Group II (analysed by one way ANOVA followed by Tukey's test).

4.3.2 Food intake

There was a significant increase in food intake per week among the HFD treated rats as compared to the normal diet-fed rats up to 8 weeks. After 8 weeks, rats treated with AEBP (300 mg/kg, p.o. and 600 mg/kg, p.o.) showed a significant decrease in food intake as compared to the HFD group. L-DOPA treated group also exhibited a significant reduction in food intake as compared to the HFD group (Fig. 4.12).

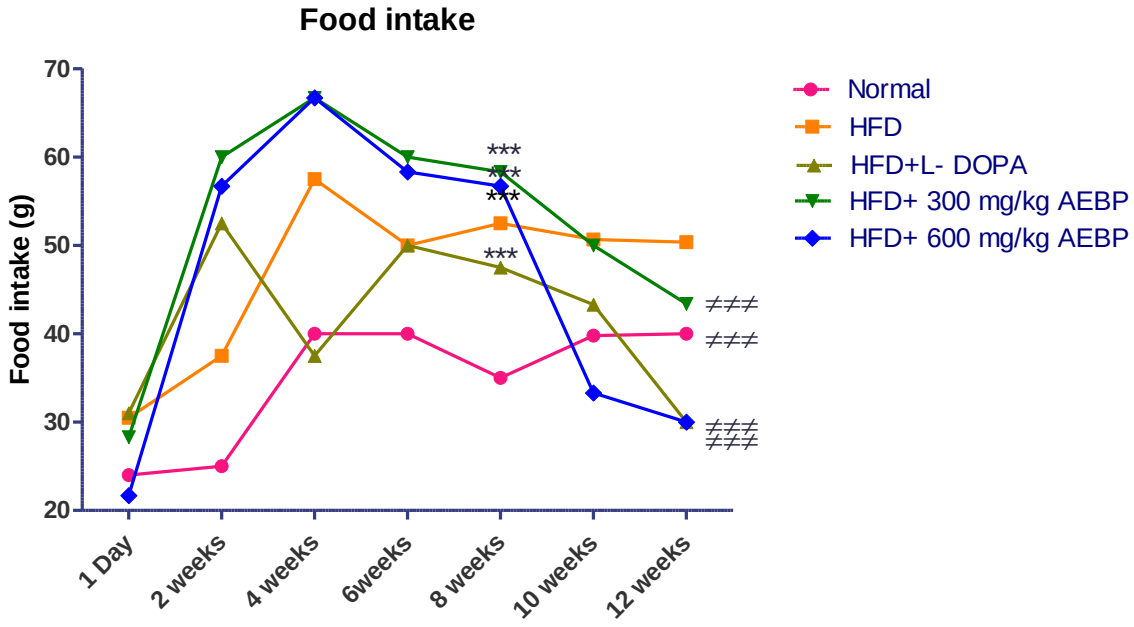


Figure 4.14: Effect of AEBP on food intake.

Each line represents values of average food intake for each group, n=6; ***p<0.001 compared to group I after 8 weeks; ###p<0.001 compared to Group II after 12 weeks (analysed by two way ANOVA followed by Bonferroni test)

4.3.3 Body mass index

HFD treated rats showed significant increase in BMI (0.84±0.05) after 12 weeks as compared to control group (0.68±0.004). L-DOPA and AEBP (600 mg/kg) treated group showed significant lower BMI (0.61±0.05 and 0.65±0.02) whereas AEBP (300 mg/kg) treated group showed no significant change in the BMI (Fig.4.13) as compared to HFD treated group.

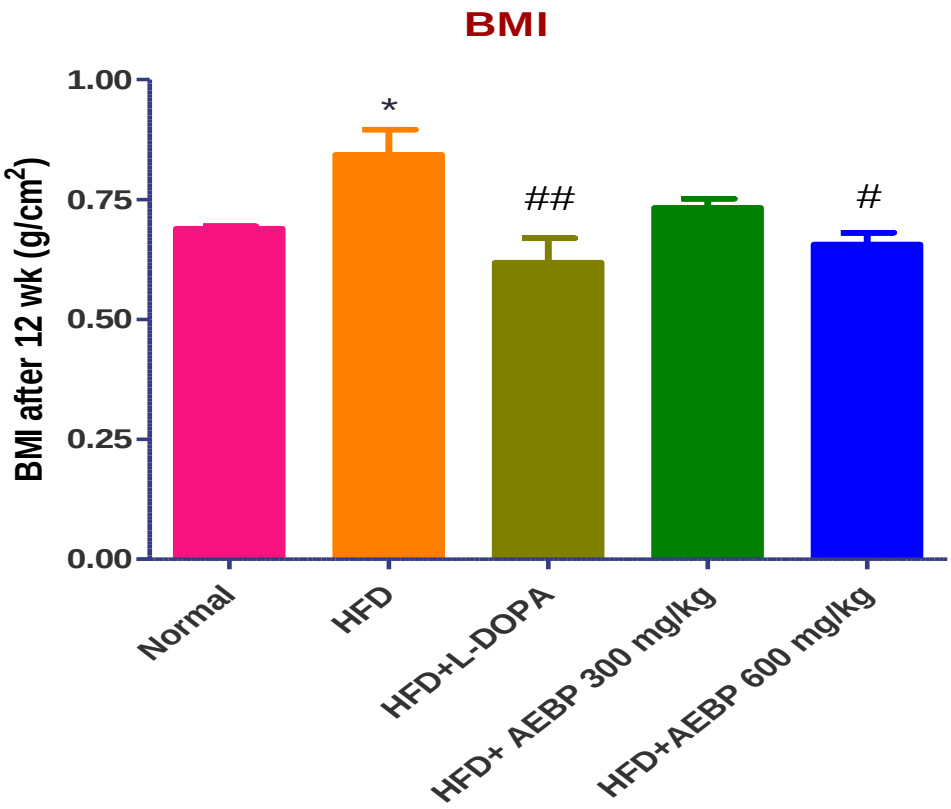


FIGURE 4.15: Effect of AEBP on BMI.

Each bar represents the mean ± SEM, n=6;*****p<0.05 compared to group I; #p<0.05, ##P<0.01 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.3.4 White adipose tissue

Feeding a high-fat diet for 12 weeks produced a significant ($p<0.05$) increase in epididymal WAT weight of HFD treated group (6.60±0.73) as compared to normal diet fed rats (3.16±0.14). There was a significant reduction in the epididymal fat mass in the L-DOPA and AEBP (600 mg/kg) treated groups (3.81±0.23 and 3.68±0.73) while no significant alteration was observed in AEBP (300 mg/kg) group as compared to HFD (Fig. 4.14) treated group.

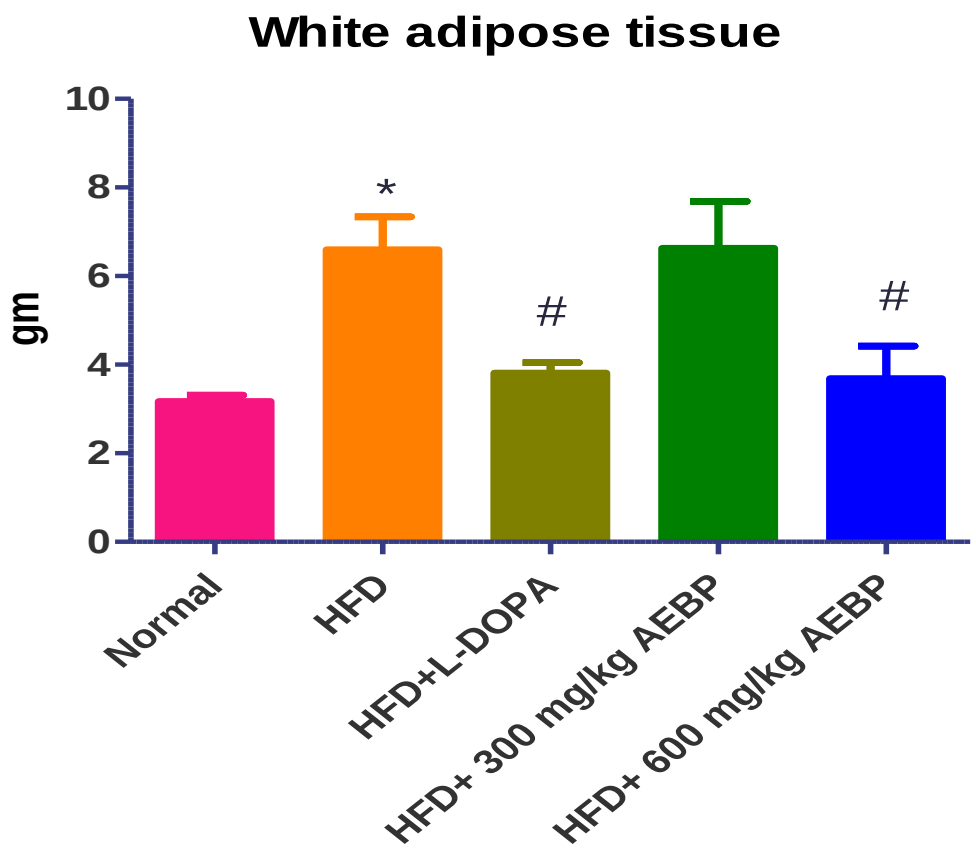


FIGURE 4.16: Effect of AEBP on white adipose tissue.

Each bar represents the mean ± SEM, n=6;*$p<0.05$ compared to group I, $^{\#}p<0.05$ compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.3.5 Serum lipid profile

Feeding of HFD caused a significant ($p<0.05$) increase in serum levels of TG as compared to normal diet fed rats. L-DOPA and AEBP (300 mg/kg and 600 mg/kg) treated groups showed significantly lower serum TG levels than in HFD group. However, no alteration was found in TC and HDL levels by treatment with either drug (Table 4.6)**.**

Table 4.6: Effect of AEBP on serum level of TG, TC and HDL (after the 12 weeks experimental period)

Serum level (mg/dl)	**Normal**	**HFD**	**HFD + L-DOPA**	**HFD + AEBP (300 mg/kg)**	**HFD + AEBP (600 mg/kg)**
TG	75.33±7.86	155.0±5.29**	118.3±4.41$^{\#}$	111.70±20.53$^{\#}$	78.67±3.84$^{\#\#}$
TC	58.67±10.37	70.67±6.489	85.67±3.33	68.00±3.78	67.33±3.84
HDL	37.93±1.67	40.33±4.48	48.70±3.2	43.30±5.83	41.47±2.57

Results are presented as means ± SEM, n=6;**p<0.01compared to normal group; $^{\#}$p<0.05, $^{\#\#}$p<0.01 compared to HFD group by one way ANOVA followed by Tukey's test.

4.3.6 Brain Dopamine levels

Brain dopamine levels of HFD group (0.12±0.006) were significantly (p<0.05) lower as compared with the control group (0.87±0.004). However, groups administered with L–DOPA and AEBP (300 mg/kg and 600 mg/kg) showed significant increase (0.82±0.03; p<0.05, 0.94±0.17; p<0.05 and 3.6±1.25; p<0.001 respectively) in brain dopamine levels as compared to the HFD group (Fig.4.15).

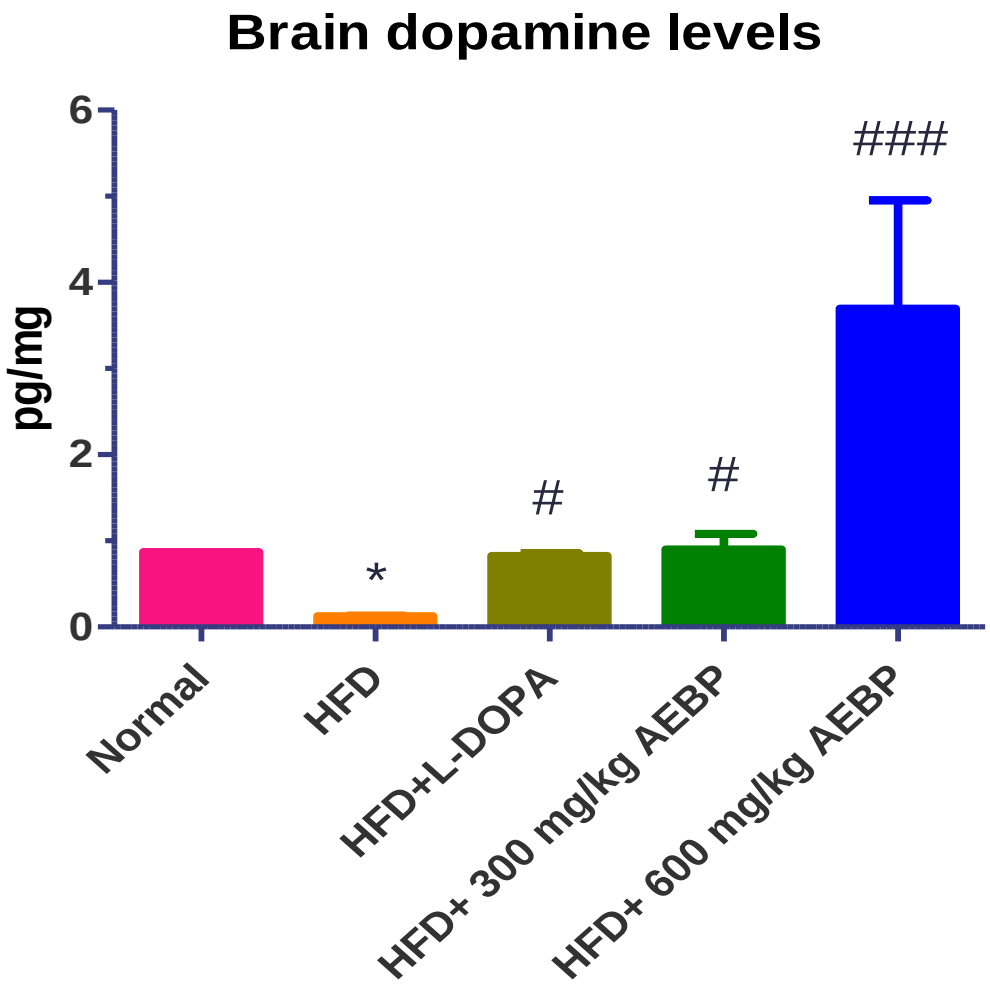


FIGURE 4.17: Effect of AEBP on brain dopamine level.

Each bar represents the mean ± SEM, n=6; *p<0.05 compared to group I, #p<0.05, ###p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.3.7 Correlation between brain Dopamine level and body weight

The graphs showed the body weight loss in all the treated animals compared to HFD group with corresponding increased brain dopamine levels

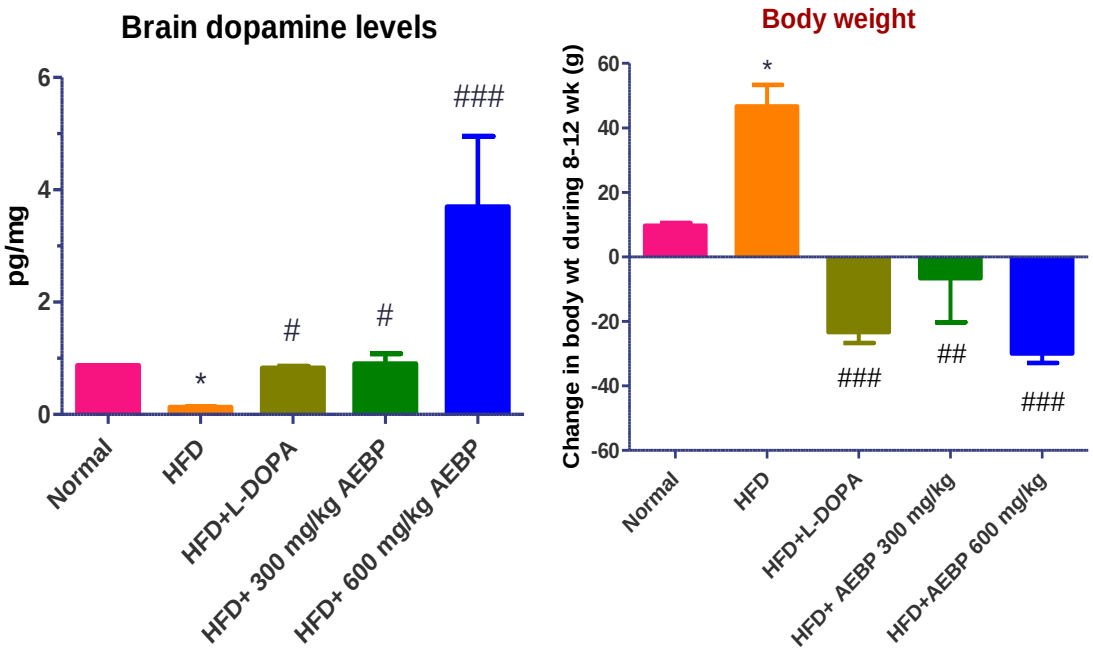


FIGURE 4.18 Brain dopamine and corresponding increased brain dopamine levels in AEBP treated group

Each bar represents the mean ± SEM, n=6; *p<0.05, compared to group I; $^{\#}$p<0.05 $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 compared to Group II (analysed by one way ANOVA followed by Tukey's test)

4.4 Phytochemical Screening

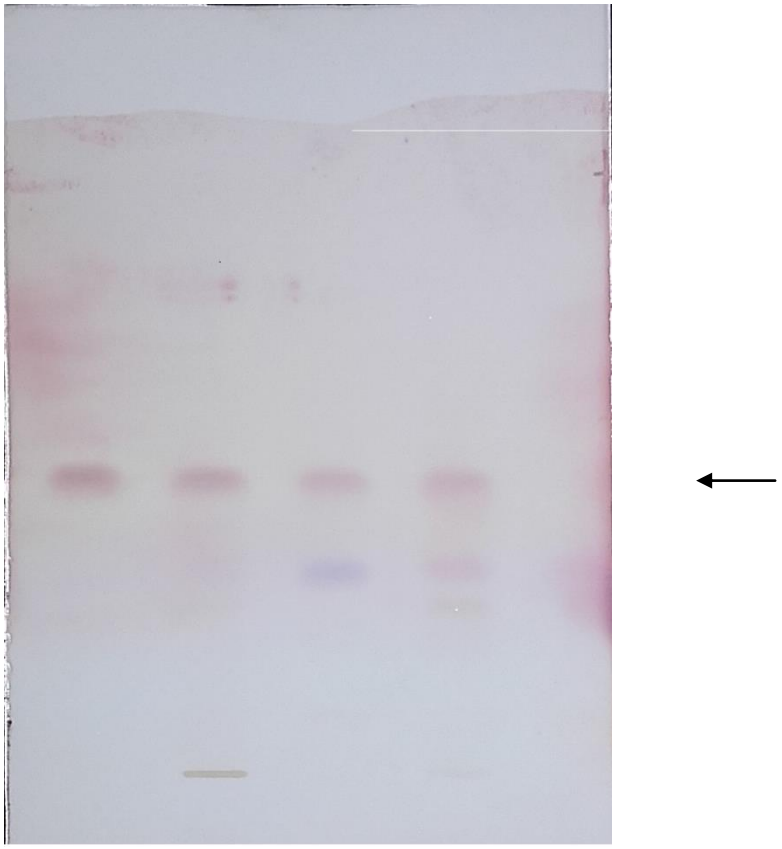
Preliminary phytochemical tests were conducted on all three plants to detect the presence of phytochemicals.

Table 4.7: Phytochemical content of all three plant seed extracts

Chemical components	Test	AEMP	AEVF	AEBP
Alkaloids	Hager's test	+	+	+
	Wagner's test	+	+	+
	Mayer's test	+	+	+
	Dragendorff's test	+	+	+
Glycosides	Molisch's test	+	-	+
	Benedicts test	+	-	+
	Fehling test	+	-	+
Carbohydrates	Molish's test	+	+	+
	Biuret test	+	+	+
Flavonoids	Shinoda test	-	+	+
	Lead acetate teat	-	+	+
Steroids	Salkwoski test	+	+	+
	Liebermann-burchard test	+	+	+
Tannins	Gelatin test	+	-	+
	Ferric chloride test	+	-	+
Saponins	Foam tets	+	+	+
	Froth test	+	+	+
Test for Aminoacid/ Protein	Ninhydrin test	+	+	+
	Biuret test	+	+	+
Starch		+	+	+

Key: + = detected - = not detected

4.4.1 TLC of all three plants extracts



A B C D

FIGURE 4.19 TLC

A: Peak of Std L-DOPA

B: Peak of *M. pruriens*

C: Peak of *V. faba*

D: Peak of *B. purpurea*

4.4.2 HPTLC fingerprinting analysis

The HPTLC analysis of L-DOPA and all three plant extracts demonstrated several peaks of different R_f values as given in table 4.8, 4.9, 4.10 and 4.11. One peak in all seed extract with R_f value 0.47 was found to be corresponding with that of std L-DOPA. This indicates that L-DOPA was one of the constituent of all seed extracts. However, there were several other prominent peaks also, indicating the presence of other constituents in significant amount.

HPTLC Chromatogram and R_f values of Std L-DOPA

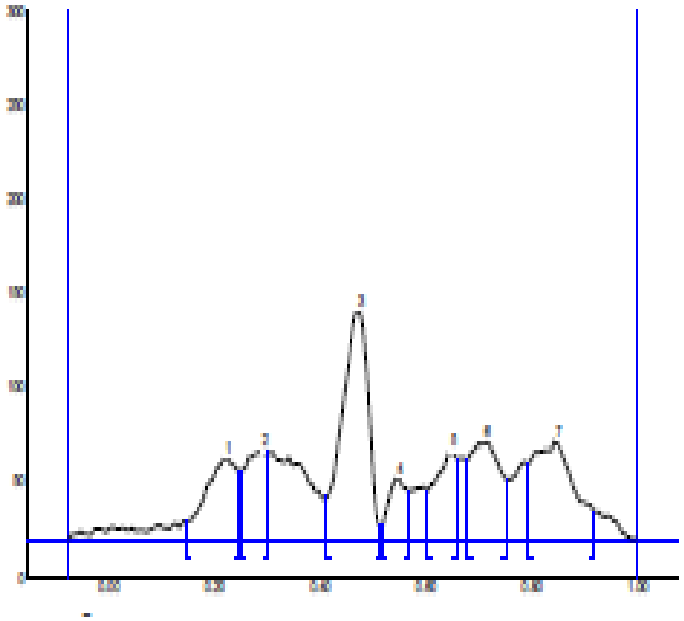


Figure 4.22: HPTLC chromatogram of Std L-DOPA

Table 4.8: Peak list and R_f values of chromatogram of Std L-DOPA at 254 nm

Peak No	Maximum R_f	Peak area	% Area
1	0.22	1950.5	12.46
2	0.29	1521.7	9.72
3	0.47	4566.8	29.17
4	0.55	873.9	5.58
5	0.65	1457.1	9.31
6	0.71	2213.3	14.14
7	0.85	3072.4	19.62

HPTLC Chromatogram and R_f values of *M. pruriens*

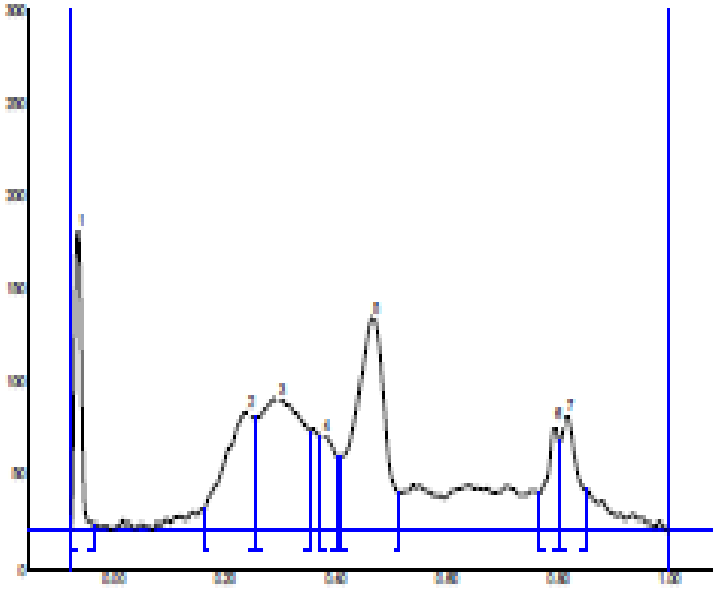


Figure 4.21: HPTLC chromatogram of *M. pruriens*

Table 4.9: Peak list and R_f values of chromatogram of *M. pruriens* extracts at 254 nm

Peak No	Maximum R_f	Peak area	% Area
1	-0.06	1566.8	9.60
2	0.24	2686.4	16.47
3	0.30	4156.7	25.48
4	0.38	1053.1	6.46
5	0.47	4556.1	27.93
6	0.80	916.7	5.62
7	0.82	1378.0	8.45

HPTLC Chromatogram and R_f values of *V. faba*

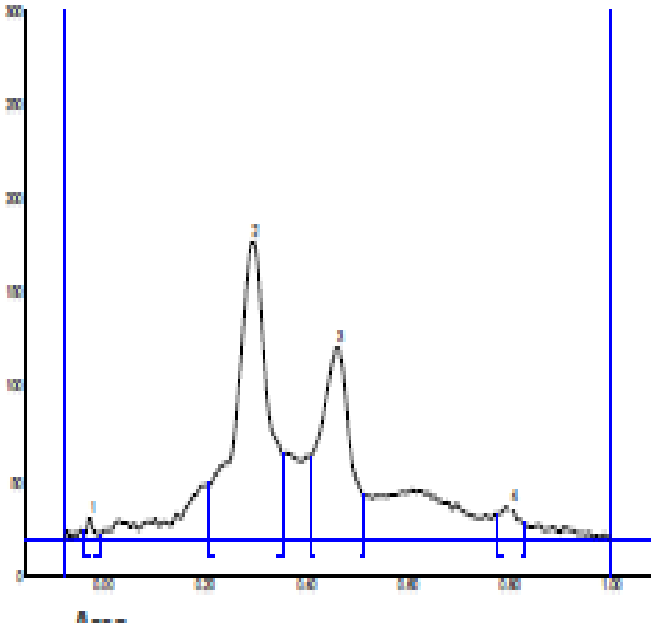


Figure 4.22: HPTLC chromatogram of *V. faba*

Table 4.10: Peak list and R_f values of chromatogram of *V. faba* extracts at 254 nm

Peak No	Maximum R_f	Peak area	% Area
1	-0.03	98.4	0.80
2	0.29	7350.6	59.76
3	0.46	4377.6	35.59
4	0.80	472.7	3.84

HPTLC Chromatogram and R_f value of *B. purpurea*

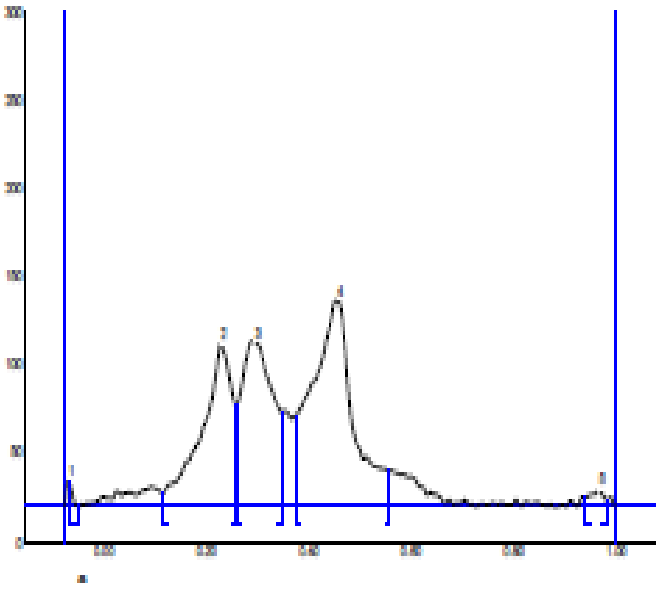


Figure 4.23: HPTLC chromatogram of *B.purpurea*

Table 4.11: Peak list and R_f values of chromatogram of *B. purpurea* extracts at 254 nm

Peak No	Maximum R_f	Peak area	% Area
1	-0.07	83.8	0.53
2	0.23	4105.8	25.76
3	0.30	4375.6	27.4
4	0.46	7176.0	45.03
5	0.97	196.5	1.23

Quantification of L-DOPA: L-DOPA concentration of aqueous extract of *M. pruriens*, *V. faba* and *B. purpurea* seeds were found to be 1.99 %, 1.91 % and 3.14 % respectively.

CHAPTER 5

Discussion

Obesity is a disorder of accumulation of excessive body fat and is caused by irregulation of energy balance (Sclafani, 2001; Gaillard et al., 2008). Multiple factors including sedentary life style, consumption of palatable food above the fulfilment of energy requirement and consumption of energy dense food are known to be involved in obesity. Also, impairment of appetite regulators like leptin, insulin and ghrelin, neurotransmitters like noradrenaline, serotonin and dopamine in brain that control the mood and behaviour towards the food also play a key role in weight management (Sahu A, 2004; Toshinai K 2003).

The role of dopamine in food reward mechanism is well known and many researches are being carried out to understand this concept (NestlerEJ and Carlezon WA, 2006; Steketee JD and Kalivas PW, 2011; Kenny PJ, 2011). However, whether the deficiencies in D_2 receptor signalling drive obesity or whether obese individuals develop deficiencies as a result of reward dysfunction is still one debate (AnirbanM, 2010). In the present study, the correlation between increased brain dopamine concentration and body weight loss is explored by studying the effects of dopamine containing plants in HFD induced obesity in rats.

Seeds of *Mucuna pruriens* are known to contain high dopamine content (L- DOPA – 1.5%) (Damodaran M et al., 1937; Modi KP et al., 2008; Paresh BS et al., 2010) and are proved to have beneficial effect in Parkinsonism in various experimental and clinical studies (Kasture SG et al., 2009; Manyam BV, 2004; Hussain G et al., 1997; Dhanasekaran S et al., 2010; Katzenschlager R, 2004). In present study also, aqueous extract of *Mucuna pruriens* seeds showed the presence of high content of L-DOPA in phytochemical analysis. Administration of 200 and 400 mg/kg of AEMP to high fat diet fed rats increased brain dopamine levels to a significant extent (1.24±0.32 and 1.081±0.06) as compared to 0.12±0.006 measured in animals fed only HFD. This was accompanied by significant mean

body weight loss of 23.33 g by administration of 400 mg/kg of AEMP, as compared to mean body weight gain of 46.67 g observed in rats fed only HFD. Administration of 200 mg/kg of AEMP for 8 to 12 weeks also prevented the rise in body weight produced by HFD and so the mean body weight gain in presence of AEMP 200 mg/kg was only 6.67 g as compared to 46.67 g with HFD alone. The effect of AEMP on body weight seems to be due to its L-DOPA content as the results were comparable to that of L-DOPA. In present study, L-DOPA caused mean body weight loss of 30 g as compared to mean body weight gain of 46.67 g with HFD alone.

The role of dopamine in controlling obesity induced by high fat diet was further confirmed by the results seen with other dopamine containing plants (*V. faba* and *B. purpurea*) in present study. Administration of aqueous extract of *V. faba* (600 mg/kg) was found to produce a mean body weight loss of 13.03 g while 300 mg/kg AEVF was found to produce mean body weight gain of only 16.67g as compared to mean body weight gain of 46.67 g with HFD alone. Similarly aqueous extract of *B. purpurea* in the dose of 300 and 600 mg/kg showed a very significant mean body weight loss of 6.67 and 30 g respectively as compared to mean body weight gain of 46.67 g with HFD alone. This was also accompanied by significant increase in brain dopamine content (0.59±0.02 and 0.67±0.004; 0.94±0.17and 3.6±1.25) with 300 and 600 mg/kg doses of AEVF and AEBP respectively as compared to 0.12±0.006 observed in HFD alone treated rats.

One of the common targets to reduce body weight gain is to control daily food intake. Also over consumption of HFD causes accumulation of adipose tissue and therefore increase in BMI. The hyperlipidaemia is also one of the determinants of obesity. Thus while controlling obesity, it is the key requirement to target such type of pathological factors that will significantly reduce the body weight or at least control further weight gain. Therefore the effects of aqueous extracts of various dopamine containing plants on other obesity parameters were also observed in the present study.

The reduction in body weight observed in all treated groups was found to be convergent with reduction in food intake observed in all treated groups as compared to the HFD group. Feeding of HFD caused a significant increase in food intake per week among the HFD treated rats as compared to the normal diet-fed rats. This is convergent with the previous reports indicating that HFD increases the food intake (Wang G et al., 2001). In the present

study, administration of aqueous extracts of *M. pruriens*, *V. faba* and *B. purpurea* from 8 to 12 weeks in addition to HFD significantly reduced average food intake as compared to HFD alone. This may be due to increase in brain dopamine content produced by these plants, since it is known that dopamine seems to regulate food intake (Martel and Fantino, 1996) by modulating food reward via the meso-limbic circuitry of the brain (Balcioglu and Wurtman, 1998). In a brain, nucleus accumbens is an important component of reward circuitry (Cone JJ et al., 2010) and the dopaminergic system is integral to reward-induced feeding behaviour (Wang G et al., 2001). This correlation between dopamine and food intake is also supported in the present study as L-DOPA was also found to significantly reduce the average food intake per week in HFD treated rats.

In L-DOPA group, reduced body weight and food intake was supported further by significant reduction of BMI, which is another important parameter of obesity. Measurement of BMI is considered to be a benchmark in classification of obesity in clinical settings. L-DOPA treated rats were found to have a significantly lower BMI (0.61± 0.051) as compared to HFD alone treated rats (0.84± 0.05) indicating the role of dopamine in preventing obesity induced by HFD. Lowering of BMI by AEMP, AEVF and AEBP although not statistically significant was found to be substantial as compared to HFD alone, while AEBP treated rats in higher dose (600 mg/kg) were found to have significantly less BMI as compared to HFD alone treated rats.

White adipose tissue mass is another important indicator of obesity. With the development of obesity, WAT undergoes a process of tissue remodeling in which adipocytes increase in both number (hyperplasia) and size (hypertrophy). Metabolic derangements associated with obesity, including type 2 diabetes, occur when WAT growth through hyperplasia and hypertrophy cannot keep pace with the energy storage needs associated with chronic energy excess. Accordingly, hypertrophic adipocytes become overburdened with lipids, resulting in changes in the secreted hormonal milieu (Sebastian D et al, 2014). In the present study, White adipose tissue mass was significantly reduced by L-DOPA, AEMP (400 mg/kg) and AEBP (600 mg/kg) but was not significantly altered in lower doses of AEMP, AEVF and AEBP treated groups. However, prevention of accumulation of adipose tissue and so adiposity was observed in all treated groups as compared to HFD alone treated group.

Obesity and overweight are accompanied by unfavourable blood lipid patterns and obesity is usually associated with higher levels of total cholesterol, LDL cholesterol and triglycerides and lower levels of HDL cholesterol (Szczygielska A et al., 2003). In the present study also, administration of HFD for 12 weeks increased serum total cholesterol and triglyceride levels significantly as compared to control animals. However administration of L-DOPA and dopamine containing plants along with HFD from 8 to 12 weeks significantly decreased serum TG levels in all treated groups with no effect on TC and HDL levels. It is reported that dopamine agonists can cause modification in TG levels via its simultaneous antilipogenic action in the liver tissue and antilipolytic action in the adipose tissue (Zhang Y et al, 1999). Thus the action of L-DOPA and dopamine containing plants on serum triglycerides observed in present study may be because of increased brain dopamine levels by these drugs.

Thus, the loss in body weight, food intake, body mass index, white adipose tissue mass and serum triglyceride levels with corresponding increase in brain dopamine levels by L-DOPA and dopamine containing plants in HFD treated rats, clearly indicate that increase in dopamine concentration can be a possible therapeutic strategy for treatment of obesity. However further studies are required in experimental and clinical settings to prove the efficacy and safety of dopamine containing plants in treatment of obesity.

CHAPTER 5

Conclusions

Results of the present study demonstrate the anti-obesity effect of aqueous extract of *M. pruriens, V. faba and B. purpurea* seeds in HFD induced obesity model.

In the present study Std L-DOPA and all the seed extracts in all doses demonstrated significant reduction in food intake. Weight reduction was observed significantly in L-DOPA and AEMP 400 mg/kg and AEBP 600 mg/kg treated rats. This was accompanied with significant increase in brain dopamine levels in these groups. Apart from this, AEBP 600 mg/kg and L-DOPA also exhibited significant reduction in BMI. White adipose tissue mass was found to be reduced by AEMP 400 mg/kg and AEBP 600 mg/kg.

Also, TG levels were significantly reduced by L-DOPA and all the plant extracts, although AEBP 600 mg/kg showed maximum benefit over others.

The results of the present study suggest that dopamine has a significant role in body weight management. However, further studies are required to investigate the efficacy profile of dopamine containing plants in obesity. In the light of the fact that till date there are no reliable drugs available for the treatment of obesity, this approach may prove to be highly beneficial in the management of obesity and related metabolic disorders.

CHAPTER 7

References

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Appendix A

Authentication certificate for plants

DEPARTMENT OF BOTANY

Faculty of Science
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA- 390 002, Gujarat, (INDIA)
Telephone: (0265) 2791891
e-mail: vinaysar@rediffmail.com
Mobile -9824244371

Dr. Vinay M. Raole
Professor

Date: 30th may .2014

To,
Mr. Javid Mansuri ,
Parul Institute of Pharmacy,
P.O. Limda, Waghodia,
Vadodara 391760

With reference to your letter No. 5821 dated November 8/4/2014 for identification of the plant specimen and submitted herbarium sheet. I would like to certify that the living plant and seed samples brought for confirmation are the same.

The samples have been identified as:

Sr. No.	Sample received as	Plant part	Sample identified as	Remark
1.	*Velvet bean*	Twig and seeds	*Mucuna puriens (L.) DC* Fam-: Fabaceae	O.K. the submitted Twig and seeds are the same as per voucher specimen.
2.	*Faba bean*	Twig and seeds	*Vicia faba L.*	O.K. the submitted Twig and seeds are the same as per voucher specimen.

With the help of submitted specimen I hereby confirm the identity as per your requirement and I hope this certificated material will be useful for your experimental work.

Wish you good luck for future endeavor.

Prof. Vinay M. Raole
PROFESSOR
DEPT. OF BOTANY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA - 390 002

सत्यं शिवं सुन्दरम्

DEPARTMENT OF BOTANY

Faculty of Science

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA- 390 002, Gujarat, (INDIA)
Telephone: (0265) 2791891
e-mail: vinaysar@rediffmail.com
Mobile -9824244371

Dr. Vinay M. Raole
Professor

Date: 26 February 2015

To,
Mr. Javid Mansuri ,
Parul Institute of Pharmacy,
P.O. Limda, Waghodia,
Vadodara 391760

With reference to your letter No. nil dated 18/2/2015 for identification of the plant specimen and submitted herbarium sheet. I would like to certify that the living plant twig and seed samples brought for confirmation are the same.

The sample has been identified as:

Sr. No.	Sample received as	Plant part	Sample identified as	Remark
1.	*Kanchnar*	Twig and seeds	*Bauhinia purpurea L.* Fam-: Caesalpiniaceae	O.K. the submitted Twig, fruits and seeds are of the same plant as per voucher specimen.

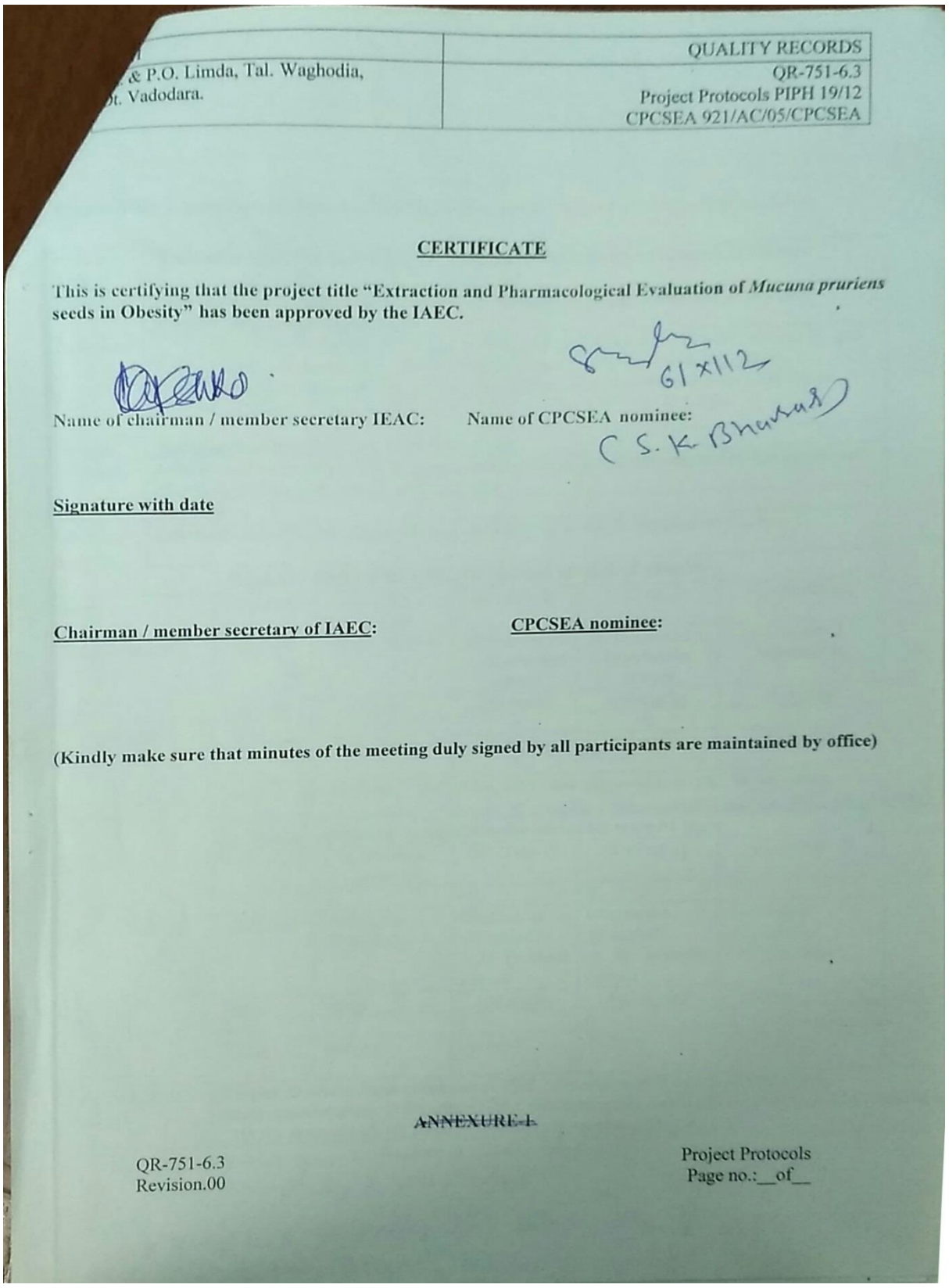
With the help of submitted specimen I hereby confirm the identity as per your requirement and I hope this certificated material will be useful for your experimental work.

Wish you good luck for future endeavor.

Prof. Vinay M. Raole
PROFESSOR
DEPT. OF BOTANY
FACULTY OF SCIENCE
MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA - 390 002

Appendix B

Certificates of approval from Institutional Animal Ethics Committee



	QUALITY RECORDS
& P.O. Limda, Tal. Waghodia, Dt. Vadodara.	QR-751-6.3 Project Protocols PIPH 19/12 CPCSEA 921/AC/05/CPCSEA

CERTIFICATE

This is certifying that the project title "Extraction and Pharmacological Evaluation of *Mucuna pruriens* seeds in Obesity" has been approved by the IAEC.

6/x/12

Name of chairman / member secretary IEAC: Name of CPCSEA nominee: (S.K. Bhutani)

Signature with date

Chairman / member secretary of IAEC: **CPCSEA nominee:**

(Kindly make sure that minutes of the meeting duly signed by all participants are maintained by office)

~~ANNEXURE-I~~

QR-751-6.3
Revision.00

Project Protocols
Page no.:__of__

PIPH At. & P.O. Limda, Tal. Waghodia, Dt. Vadodara.	QUALITY RECORDS QR-751-6.3 Project Protocols PIPH 12/14 CPCSEA 921/AC/05/CPCSEA

CERTIFICATE

This is certifying that the project title "Pharmacological Evaluation of dopamine containing plants in Obesity" has been approved by the IAEC.

Name of chairman / member secretary IEAC: **Name of CPCSEA nominee:**

Signature with date 18/1/14

18/I/14
(S. K. Bhansali)

Chairman / member secretary of IAEC: **CPCSEA nominee:**

(Kindly make sure that minutes of the meeting duly signed by all participants are maintained by office)

QR-751-6.3
Revision.00

Project Protocols
Page no.: 6 of 8

PIPH At. & P.O. Limda, Tal. Waghodia, Dt. Vadodara.	QUALITY RECORDS QR-751-6.3 Project Protocols PIPH 01/15 CPCSEA/921/PO/ERe/S/05/CPCSEA

CERTIFICATE

This is certifying that the project title "Pharmacological Evaluation of dopamine containing plantsin experimentally induced Obesity"has been approved by the IAEC.

Name of chairman / member secretary IEAC: **Name of CPCSEA nominee:**

Signature with date

18/6/15

Chairman / member secretary of IAEC: **CPCSEA nominee:**

(Kindly make sure that minutes of the meeting duly signed by all participants are maintained by office)

List of publications

1) Mansuri J, Pithadia A, Navale A, Shetty R, Paranjape A. Clinical manifestation of Obesity. Pharmagene, 2013; 1(1):65-69.
2) Evaluation of anti-obesity effect of aqueous extract of *Mucuna pruriens* seeds on rats. Int J Pharm Pharm Sci, 2017; 9(3):111-115.