Chapter I

INTRODUCTION
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Obesity, the most common nutritional disorders in humans, is becoming a major problem not only in Asia but almost all over the world (Marinou et al., 2010). According to WHO, the prevalence of obesity is rapidly rising to epidemic proportion around the world at an alarming rate. More than 1.6 billion adults worldwide are overweight and at least 400 million are obese a figure which is projected to almost double by 2015 (Herber, 2010). In North America and most European countries, the obesity rates have more than doubled in the last 20 years (Elangbam, 2009). The International Obesity Task Force estimates that more than 300 million individuals worldwide are obese body mass index (BMI $\geq 30$ kg/m$^2$) and 800 million are overweight (BMI between 25 and 29.9 kg/m$^2$). Currently, 66% of US adults are overweight or obese, 16% of US children and adolescents are overweight, and 34% are at risk of becoming overweight (Haslam and James, 2005).

The obesity epidemic is not restricted to industrialized societies alone; this increase is often faster in developing countries than in the developed world. Obesity and overweight pose a major risk for serious diet-related chronic diseases, including type 2 diabetes, hyperlipidemia, cardiovascular disease, hypertension and stroke, obstructive sleep apnea, asthma, orthopedic disorders, social and mental health problems and certain forms of cancer (WHO, 2003).

Obesity can be defined as syndrome characterized by an increase in body fat stores, mainly due to an imbalance between energy intake and energy expenditure. Obesity results when energy intake exceeds energy expenditure. The global epidemic of obesity results from a combination of genetic susceptibility, increased availability of high energy foods and decreased physical activity in modern society. Obesity should no longer be regarded simply as a cosmetic problem affecting certain individuals (obese individuals are often stigmatized socially), but an epidemic that threatens global well being (Kopelman, 2000). The primary cause of obesity lies in environmental and behavioral changes, although genetic factors contribute to the
propensity of an individual to become obese. One well-established important environmental factor predisposing to obesity is the amount of fat in the diet. Epidemiological studies have identified a significant positive correlation between average dietary fat intake and the incidence of obesity (Kuller, 1997). The WHO states that obesity is one of the most blatantly visible, yet most neglected, public health problems. Recent data suggest that the waist-to-hip ratio may even better predict cardiovascular illnesses than BMI (Yusuf et al., 2005).

Obesity is defined by body mass index (BMI). The BMI is body mass or weight (kg) divided by the square of the height (meters), it is highly correlated with body fat. World Health Organization (WHO) Expert Committee (1995) for the classification of overweight and obesity proposes BMI of 30 kg m\(^{-2}\) or greater as (Grade 2 overweight) obesity, but this do not take into account the detrimental effect of intra-abdominal fat. Extensive research into obesity has shown the location of body fat deposits rather than their size is more important in determining the risk of developing obesity linked disorder (Björntorp, 1997). In fact, the accumulation of intra-abdominal visceral fat in the mesentery and omentum is a better predictor of coronary heart disease than the body mass index (Nakamura et al., 1994).

The measurement of fat distribution has become an important issue in obesity research. Numerous techniques have been developed to assess visceral fat because this fat seems to be the most strongly associated with metabolic disorder. Magnetic resonance imaging is one of the optimal techniques available for measuring the visceral fat (Sobol et al., 1991). Anthropometric measurements can be useful to classify subjects into different types of fat distribution for diagnosis of abdominal obesity and for general application in epidemiological studies.

The association between hypertension and obesity is well documented (Zhang and Reisin, 2000). A cross-sectional study of the general population showed that every 10 kg increase in body weight was associated with an increase of 3 and 2 mm Hg in systolic and diastolic pressures, respectively (Boe et al., 1957).
Leptin is a circulating hormone that is expressed abundantly and specifically in adipose tissue, although it is also secreted from human placenta. Leptin induces a complex response involving control of body weight and energy expenditure. Leptin measurement is an important index of obesity. Leptin levels increase exponentially with increasing fat mass and leptin production is higher in subcutaneous than in visceral fat depots (Auwerx et al., 1998; Lonnqvist et al., 1997; Considine et al., 1996). Leptin levels reflect not only the amount of fat stored but also energy imbalance; prolonged fasting substantially decreases leptin levels, whereas overfeeding greatly increases these levels (Kolaczynski et al., 1996).

An association between adiposity and insulin resistance has been reported in adults and children. Weight loss is associated with a decrease in insulin concentration and an increase in insulin sensitivity in adults (Su et al., 1995). In a study of 122 adolescents, obese individuals were significantly more insulin resistant and had an abnormal lipid profile when compared with lean subjects (Steinberger et al., 1995).

One important pathologic phenomenon associated with obesity and its related conditions is cardiomyocyte cell death, and in particular, cardiomyocyte apoptosis (Trivedi and Barouch, 2008). Obesity is often associated with hemodynamic overload, ventricular remodeling, and higher cardiac output due to an augmented stroke volume and an increase in heart rate (Alexander, 1993). Obesity cardiomyopathy typically occurs in persons with severe and long-standing obesity, which may progressively develop congestive heart failure and sudden cardiac death (Alpert, 2001). It has been reported that high fat diet induces apoptosis (Wang et al., 2008).

Activation of cysteine proteases called caspases plays a major role in the execution of apoptosis. These proteases selectively cleave vital cellular substrates, which results in apoptotic morphology and internucleosomal fragmentation of DNA by selectively activated DNases. Mitochondrial dysfunction causes release of cytochrome c which binds to Apaf-1 in the presence of ATP and further promotes activation of procaspase 9 and then to caspase 3 (Haunstetter and Izumo, 2000). Internucleosomal
fragmentation of genomic DNA has been the biochemical hallmark of apoptosis for many years (Wyllie, 1980). The first method used to detect apoptosis associated DNA fragmentation, was by ethidium bromide staining of total genomic DNA that was size fractionated on an agarose gel (Van den Hoff et al., 2000). The obtained results show a characteristic ladder of DNA bands with increasing size as a result of internucleosomal DNA fragmentation.

Blood lipid levels are often abnormal in obese persons. High-density lipoprotein (HDL) cholesterol, a higher level of which has been clearly implicated in decreased risk for coronary heart disease, is lower in obese persons (Glueck et al., 1980). Total and low-density lipoprotein (LDL) cholesterol, however, have been found in cross-sectional studies to be normal (Montoye et al., 1966) or elevated in obese compared with lean persons (Assman and Schulte, 1992).

Apolipoproteins provide the structural element to the lipoprotein particles as well act as ligands for specific enzymes and receptors in lipoprotein metabolism. The apolipoproteins are important determinants of the metabolism and structure of plasma lipoproteins. Measurement of the levels of the apo A1 and apo B can throw light on lipid metabolism. Apolipoprotein A1 is a component of high density lipoproteins (HDL) and activator of plasma lecithin/cholesterol acyltransferase. Apolipoprotein B contains ligands for the receptor-mediated endocytosis of lipoproteins and is an essential component of chylomicrons and low density lipoproteins (LDL). There is an inverse relationship between apo A1 and coronary artery disease (CAD) and a direct relationship with apo B such that patients with CAD have generally reduced levels of apo A1 and increased levels of apo B (D'Souza et al., 2007).

The sodium (Na) and potassium (K) activated adenosine-triphosphatase (Na, K-ATPase) is a membrane enzyme that energizes the Na-pump by hydrolyzing adenosine triphosphate and wasting energy as heat, so playing a role in thermogenesis and energy balance. Animal and human obesity is associated with reduction of tissue Na\(^+\) K\(^+\) ATPase, linked to hyperinsulinemia (Iannello et al., 2006).
Obesity has been shown to be one of the conditions that decrease antioxidant capacity. Obesity decreases antioxidant defense by lowering the levels of antioxidant enzymes (catalase, glutathione peroxidase (GPx) and glutathione reductase (GRd)) (Carmiel-Haggai et al., 2005; Asayama et al., 2001).

Obesity that develops in response to the availability of variety of palatable, high fat, high carbohydrate foods in the animal models (Sclafani and Springer, 1976) may be most closely resembles human diet-induced obesity. The molecular basis of these obesity-related changes is poorly understood. A high-fat diet (HFD) has been reported to adversely affect the health of humans and animal species (Ghosh et al., 2001). Chronic consumption of a high-fat diet induces obesity, insulin resistance, dyslipidemia, and type 2 diabetes (Shin et al., 2008). It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity (Slim et al., 1996). Recent studies have shown that consumption of dietary fats promotes hypothalamic resistance to the main anorexigenic hormones, leptin and insulin, leading to the progressive loss of the balance between food intake and thermogenesis and therefore, resulting in body mass gain. A high-fat diet, induces BP elevation, could derange the neurohumoral control of the kidney (Hall et al., 1993). More fat is stored and the individual moves along the scale toward obesity.

For the first time, leading global obesity and diabetes organizations have come together to provide recommendations for stemming the twin epidemics which threaten to explode in the coming decade. It is estimated that about 80% of type 2 diabetes patients are obese (Ehtesham, 2001). Obesity is associated with an increased risk of developing insulin resistance and type 2 diabetes. There is a greater prevalence of diabetes found among obese people compared to those with normal weight (Steinberger and Daniels, 2003). Indeed, obesity particularly that involving an increase in fat deposits within the abdomen (visceral obesity) and upper body is an independent risk factor for non-insulin dependent diabetes mellitus (NIDDM).

Insulin resistance, working through hyperinsulinemia, also affects the very complex control of fat metabolism. These disturbances lead more or less directly to the typical
pattern of dyslipidemia seen in obesity and NIDDM: hypertriglyceridaemia; low HDL-cholesterol levels; and to a lesser extent high LDL. These are all known risk factor for coronary artery disease and other macrovascular complications. In addition, essential hypertension like obesity and NIDDM can be caused by an insulin resistant state (Ginsberg et al., 2006).

Currently approved drugs for long term treatment of obesity includes sibutramine, which inhibits food intake and orlistat which blocks fat digestion. Current drugs for treatment of obesity have a useful place as part of the treatment programme for some overweight patients. But because obesity is worldwide epidemic, there is a major need for more medicinal products that are proven safe and effective when used as prescribed. In addition, as obesity is associated frequently with other disorders such as diabetes, hypertension, dyslipidemia, sleep apnoea and osteo-arthritis, it is important that the physician treat all of the relevant treatable diseases (Bray and Tartaglia, 2000).

For the scientist, the challenge of obesity is to identify those major pathways where pharmacological intervention can safely and effectively help those who are clinically overweight.

Due to obscure aetiology, the treatment of obesity is difficult and challenging. Further, the cause of concern is the non-availability of drugs for its treatment and the short-term efficacy and limiting side effects of the available drugs (Kaur and Kulkarni, 2000). There are at present, no totally effective, safe drugs for the prevention of obesity, but the understanding of the basis of obesity is progressing rapidly. For a drug to have significant impact on body weight, it must ultimately reduce energy intake, increase energy expenditure or both.

Recent awareness of therapeutic potential of several traditionally used plants has opened a new dimension for the study and research of medicinal plants. In fact, the WHO has estimated that 80% of world's population that is over 4 billion people at least partially uses herbal medicine for meeting their health care needs. In India,
China and Far East countries, products are available based on traditional systems, both in their native forms produced and dispensed by traditional physicians and also as modern versions such as tablets, capsules etc.

Due to its complex and multifactorial pathogenesis of obesity, behavioral therapy alone has had limited success in providing meaningful, sustained weight reduction, and pharmacological treatment becoming a challenging research priority. And many such studies are being carried out to detect and confirm the action of drugs and natural products that yield better and long-lasting effect in term of weight reduction. In this field, medicinal plants play a very important role.

Phytatherapy is becoming increasingly popular both for the results, it yields in several pathologies, and for a growing sense of mistrust towards conventional medical treatments. It seemed, therefore, reasonable and timely to assess the validity of phytatherapeutic products in the treatment of obesity (Moro and Basile, 2000).

*Gymnema sylvestre* is an Indian herb, known as Gurmar i.e. sugar destroyer. Gymnema leaves have been used for centuries in the traditional Indian system of Ayurvedic medicine. The term “destroyer of sugar” is traditionally used for Gymnema because chewing the leaves will abolish the taste of sweetness. The medicinally active parts of the plant are the leaves and the roots. Recent clinical trials conducted in India have shown that an extract of *Gymnema sylvestre* is useful for controlling blood sugar (Baskaran et al., 1990).

The presence of Gymnemic acid (GA), (+) quercitol, lupeol, β-amyrin, stigmasterol etc., has been reported in *Gymnema sylvestre*. GA I, II, III and IV are anti sweet substances from the leaves of *G. sylvestre*. They all contain a glucuronic acid moiety, and the gymnemagenin aglycone esterified at position C-21 and C-28. A second series of Gymnemic acid V-VII has also been reported. Gurmarin, another constituent of the leaves, and gymnemic acid have been shown to block sweet taste in humans. Some researchers have suggested gymnemic acid as one possible candidate responsible for antidiabetic activity.
Gymnema sylvestre R. Br. (family: Asclepiadaceae) is a native plant in the south west of India, Australia and tropical Africa. From ancient times, G. sylvestre has been used in Indian traditional medicine ("Ayurvedic medicine") and is considered to be effective in improving urination, stomach stimulation, and diabetes (Nadkarni, 1982). Another useful study showed that Gymnema helped reverse the pathological changes occurring in the liver, kidney, and muscles as a result of hyperglycemia (Okabayashi et al., 1990). Bishayee and Chaterjee (1994) reported the hypolipidemic and antiatherosclerotic effect of oral Gymnema sylvestre leaf extract in albino rats fed on high fat diet. The ethanol extract of Gymnema sylvestre leaves showed an antimicrobial activity (Satdive et al., 2003). Standardized G. sylvestre extract in combination with niacin-bound chromium (NBC) and hydroxycitric acid (HCA-SX) has been evaluated for antiobesity activity in moderately obese subjects (Preuss et al., 2004). Gymnema sylvestre leaves extract has anti-inflammatory activity in rats (Malik et al., 2008). Rachh et al. (2010) reported that Gymnema sylvestre R. Br. leaves extract has antihyperlipidemic activity in rats fed with high cholesterol diet. If the drug under investigation modulates the carbohydrate and lipid metabolism, it can be further investigated for its beneficial activity in obesity related disorders.

Streptozotocin is a well documented diabetogen to induce diabetes mellitus (NIDDM) in experimental models (Nakhaee et al., 2009). Diabetic rats when fed with high fat diet produce a metabolic syndrome characterized by insulin resistance, dyslipidemia, type-2 diabetes and central obesity, which is similar with the metabolic syndrome caused by obesity. As Carbohydrate and lipid metabolisms are closely linked processes, derangement in the carbohydrate metabolism produces dyslipidemia, hence, STZ + HFD model is one of the ideal model for screening of antiobesity activity in diabetic rats.

This statement is supported by the findings of Ding et al. (2005) who reported that Wistar rats injected intraperitoneally with low dose of STZ (30 mg/kg) and fed with a high sucrose-fat diet for 8 weeks, develop significant insulin resistance and obesity. Similarly, Srinivasan et al. (2005) reported that high fat diet –fed and low dose of
STZ (35 mg/kg, i.p.) treated rats simulate natural disease progression and metabolic characteristics typical of individuals at increased risk of developing type 2 diabetes because of insulin resistance and obesity. Further, Zhang et al. (2008) demonstrated that a combination of HFD and low dose of STZ (45 mg/kg) injection effectively used to generate a rat model that mimics the natural history and metabolic characteristics of type 2 diabetes in humans.

The present study was designed to investigate the antiobesity potential of *Gymnema sylvestre* extract in high fat diet-induced obesity and associated metabolic disorders using normal healthy Wistar rats and streptozotocin induced diabetes in obese Wistar rats.