

**EFFECT OF ETHANOLIC EXTRACTS OF *ANDROGRAPHIS PANICULATA*
ON TYPE 2 DIABETES MELLITUS AND INSULIN RESISTANT RATS**

by

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**This is submitted in fulfillment of the
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This thesis is dedicated to ...

My Parents, Brother and sister-in-law for their constant love, support and encouragement

and

To Lord Vinayaga

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LIST OF ABBREVIATIONS AND SYMBOLS

% = percentage

°C = degree centigrade

µg/ml = microgram per milliliter

µl = microlitre

Acetyl-CoA = Acetyl coenzyme

ACP = Acid phosphatase

ADA CPR = American Diabetes Association Clinical Practice Recommendations

ADP = Adenosine diphosphate

ALP = Alkaline phosphatase

AP = *Andrographis paniculata*

AG = Andrographolide

ANOVA = Analysis of variance

ATP = Adenosine triphosphate

AUC = Area under the curve

BG = Blood glucose

B.wt = body weight

BSA = Bovine serum albumin

cAMP = cyclic Adenosine monophosphate

CB-1 = Cannabinoid receptor-1

CCl₄ = Carbon tetrachloride

DNA = Deoxyribonucleic acid

CDC = Centre for Disease Control

CV = Coefficient of variation

DeAG = 14-deoxy-11,12-didehydroandrographolide
DNA = Deoxyribonucleic acid
DPP-IV = Dipeptidyl peptidase-IV
DW = Distilled water
FDA = Food and Drugs Administration
FFA = Free fatty acids
FPG = Fasting plasma glucose
FSIVGTT = Frequently sampled intravenous glucose tolerance test
GIP = Glucose-dependent insulintropic polypeptide
GLP-1 = Glucagon-like peptide-1
GLUT 4 = Glucose transporter 4
GK = Glucokinase
G6P'Tase = Glucose 6 phosphatase
G6PDH = Glucose 6 phosphate dehydrogenase
GM-CSF = Granulocyte Macrophage-Colony Stimulating Factor
GOT = Glutamate oxaloacetate transaminase
GPT = Glutamate pyruvate transaminase
GPx = Glutathione peroxidase
GR = Glutathione reductase
GSH = Glutathione S hydroxylase
GST = Glutathione S tranferase
HbA_{1c} = Hemoglobin A_{1c}
HK = Hexokinase
HPLC = High Performance Liquid Chromatography
HPTLC = High Performance Thin Layer Chromatography

Hrs = Hours

HDL = High density lipoprotein

HOMA = Homeostasis model assessment

IFN- γ = Interferon-gamma

IgG = Immunoglobulin G

IGT = Impaired glucose tolerance

IRC = Insulin resistance control

IL-1 β = Interleukin-1-beta

IL-6 = Interleukin-6

ip = Intra peritoneal

IPGTT = Intra peritoneal glucose tolerance test

IRS-2 = Insulin receptor substrate-2

LDH = Lactate dehydrogenase

LP = Lipid peroxidation

LDL = Low density lipoprotein

LOD = Limit of detection

LOQ = Limit of quantitation

mg = milligram

μ g = microgram

mNADH = mitochondrial NADH

mRNA = messenger ribonucleic acid

NAD⁺ = Oxidised NAD

NADH = Nicotinamide adenine dinucleotide

NDFS = National Diabetes Fact sheet

NeAG = Neoandrographolide

NO = Nitric oxide

NC = Normal control

NPH = Neutral Protamine Hagedorn

OGTT = Oral glucose tolerance test

PBG = Peak blood glucose

PDM = Persetuan Diabetes Malaysia

po = peroral

PG = Pioglitazone

PKC- β = protein kinase C-beta

PKC- ϵ = Protein kinase C-epsilon

PPAR- γ = Peroxisome proliferator activated receptor- gamma

PPG = Post prandial glucose

PTP-1B = protein tyrosine phosphatase 1B

ROS = Reactive oxygen species

RP-HPLC = Reversed phase High performance liquid chromatography

RSD = Relative standard deviation

SEM = Standard error of mean

SPSS = Statistical procedures for social sciences

STZ = streptozotocin

STZ-NA = streptozotocin-nicotinamide

sc = subcutaneous

T2DM = Type 2 Diabetes Mellitus

TBARS = Thiobarbituric acid reacting substances

TIMP-1 = Tissue inhibitor of metalloproteinase-1

Trtmnt = Treatment

TNF- α = Tumour necrosis factor- alpha

USP DI = United States Pharmacopoeia Drug information

UV-VIS = Ultraviolet-Visible

VEGF = Vasculoendothelial growth factor

WHO = World Health Organisation

KESAN EKSTRAK ETANOL *ANDROGRAPHIS PANICULATA* KEATAS TIKUS DIABETIS MELLITUS JENIS 2 DAN RINTANG INSULIN

ABSTRAK

Kebanyakan kajian mengenai aktiviti antidiabetik *Adrographis paniculata* sebelum ini termasuk yang dilakukan oleh Kasmuri (2006) tertumpu kepada kesan tumbuhan tersebut keatas diabetis jenis I sedangkan lebih dari 98 % pesakit diabetis adalah dari jenis 2 (T2DM). Oleh itu, tujuan kajian ini adalah untuk menilai kesan-kesan ekstrak etanol *A. paniculata* keatas tikus T2DM dan rintang insulin.

Percubaan *in vitro* perencatan enzim alfa glukosidase dan alfa amilase menunjukkan ekstrak etanol mempunyai kesan perencatan aktiviti alfa glucosidase yang nyata dan perencatan alfa amilase yang lemah. Penemuan kajian ini disokong oleh ujian perencatan aktiviti alfa glukosidase akut *in-vivo* pada kedua-dua tikus normal dan tikus diabetik. Rawatan dengan ekstrak etanol 250,500 dan 1000 mg/kg menyebabkan penurunan puncak glukosa (PBG) dan kawasan di bawah keluk (AUC) dan oleh itu boleh merencat peningkatan PBG bila diberikan cabaran dengan kanji dan sukrosa.

20%v/v 250,500, dan 1000mg/kg dan ekstrak etanol 95% v/v 500 dan 1000 mg/kg tambahan pula, rawatan kronik dengan ekstrak etanol selama 21 hari dapat menurunkan paras glukosa serum tikus yang diaruhkan T2DM menggunakan nikotinamida dan streptozotosin. Didapati berlaku penurunan paras enzim glukoneogenik dan peningkatan paras enzim glikolitik dan lipogenik hati. Paras kolesterol, trigliserida, dan asid lemak bebas serum juga menurun. Disamping itu berlaku juga peningkatan kecil paras glutatation S hidroksilase (GSH), glutatation S transferase (GST), dan glutatation reduktase (GR) hati diperkirakan akibat dari kesan

antioksidatif. Keputusan percubaan menyarankan pemberian ekstrak telah menekan glukoneogenesis dan glikogenesis dan seterusnya meningkatkan glikolisis dan glikogenesis.

Akhirnya kesan pemberian ekstrak secara keronik selama 30 hari dinilai keatas tikus rintang insulin aruhan diet lemak dan streptozotosin. Pemerhatian menunjukkan ekstrak etanol 20%v/v 500, dan 1000mg/kg ekstrak etanol dan 95% v/v 500 dan 1000 mg/kg tambahan pula boleh menyegerakan pelupusan glukosa dari darah dan mengurangkan peningkatan AUC glukosa. Dalam ujian cabaran dengan tolbutamida, ekstrak didapati berupaya meningkatkan tindakbalas terhadap glukosa serum. Kesan kehilangan gerak balas terhadap tolbutamida telah di lambatkan pada tikus yang dirawat dengan ekstrak dan seterusnya melambatkan berlakunya kerintangan terhadap insulin. Kesan peningkatan kepekaan terhadap insulin oleh pemberian kronik ekstrak selama 30 hari telah dinilai pada tikus diabetik aruhan streptozotosin. Keputusannya didapati pemberian ekstrak etanol 20%v/v tidak memberikan kesan terhadap kepekaan insulin, tetapi pemberian ekstrak etanol 95%v/v telah meningkatkan dengan sederhana kepekaan terhadap insulin sepertimana kesan metformin.

Oleh itu aktiviti antidiabetik boleh diperantarakan secara bebas oleh mekanisme ekstra pancreas seperti yang ditunjukkan oleh bertambah baiknya paras glukosa semasa berpuasa akibat dari perencatan glukosidase, perangsangan penggunaan glukosa perifer dengan memudahkan oksidasi dan penggunaannya, peningkatan kepekaan terhadap insulin atau kombinasi dari semua faktor diatas. Penemuan yang menggalakan ini menjadikan ekstrak etanol *A. paniculata*

berpotensi digunakan untuk merawat T2DM dan keadaan rintang insulin pada manusia.

EFFECT OF ETHANOLIC EXTRACTS OF *Andrographis paniculata* ON TYPE 2 DIABETES MELLITUS AND INSULIN RESISTANT RATS

ABSTRACT

Most of the previous studies on anti-diabetic activity of *Andrographis paniculata* including the studies performed by Kasmuri (2006) were concentrating on the effect of the plant on Type-I diabetes whereas more than 98% of diabetics in the affected population are of the type 2 (T2DM) nature. Therefore, the aim of the present study was to evaluate the effects of ethanolic extracts of *Andrographis paniculata* on T2DM and insulin resistance rats.

In vitro alpha glucosidase and alpha amylase enzyme inhibitory experiments demonstrated that both 20%v/v and 95% v/v ethanolic extracts have appreciable alpha glucosidase and a weak alpha amylase inhibitory activity. This finding was further supported by acute *in vivo* alpha glucosidase inhibitory test in diabetic rats. The ethanolic extracts treatment at doses of 250, 500, and 1000mg/kg caused a reduction in peak blood glucose (PBG), and area under the curve (AUC) levels and thus could inhibit the rise in PBG when challenged with starch, and sucrose.

Furthermore, chronic treatment for 21 days with 250, 500 and 1000mg/kg of 20%v/v ethanol extract and and 500, 1000mg/kg of 95% v/v ethanolic extracts reduced the fasting serum glucose levels of nicotinamide and streptozotocin-induced T2DM rats. There was a reduction in liver gluconeogenic enzyme level and an increase in liver glycolytic and lipogenic enzyme levels on treatment with 20%v/v ethanolic extract. The serum cholesterol, triglycerides, and free fatty acids were also reduced with both the extracts at all the doses. There was also a small increase in liver glutathione S hydroxylase (GSH), glutathione S transferase (GST), and

glutathione reductase (GR) enzyme activity suggesting an anti-oxidative effect. The results suggest that the 20% ethanolic extract may suppress gluconeogenesis and glycogenolysis with a subsequent increase in glycolysis and glycogenesis.

Finally the effects of chronic treatment for 30 days with the extracts were evaluated in a fat-fed and low dose streptozotocin-induced insulin resistance rats. It was observed that the 500 and 1000 mg/kg doses of 20% v/v and 1000mg/kg dose of 95% v/v ethanolic extract hastened the blood glucose disposal and reduced incremental glucose AUC. In the tolbutamide challenge test the same doses of both the extracts were observed to cause an increase in the serum glucose response. The loss of response to tolbutamide was delayed in extract treated groups thus effectively delaying insulin resistance. The insulin sensitising effect of the 30 days chronic treatment of the extracts was evaluated in streptozotocin-induced diabetic rats. It was found that 500 and 1000mg/kg doses of 20%v/v ethanolic extract had no insulin sensitising effect, while the 1000mg/kg dose of 95%v/v extract demonstrated a moderate insulin sensitising effect like metformin.

Thus the anti-diabetic activity could be mediated independently by an extra pancreatic mechanism as shown by distinct improvement in fasting blood glucose levels due to alpha glucosidase inhibition, stimulation of peripheral glucose utilization by facilitating oxidation and utilization, increasing insulin sensitivity or by a combination of all the above mechanisms. These promising findings make the ethanolic extracts of *Andrographis paniculata*, a good potential in the treatment of T2DM and insulin resistance cases in humans.

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CHAPTER 1

Introduction

1.0. Type 2 Diabetes Mellitus and Insulin Resistance

Type 2 diabetes mellitus (T2DM) is a progressive, chronic metabolic disorder notable for the underlying defects in carbohydrate and lipid metabolism. It is typically characterized by several sequential steps involving impaired beta cell function, resulting in a relative insulin deficiency, followed by insulin resistance with decreased glucose transport into muscle and fat cells, accompanied by unrestrained hepatic glucose output, all of which contribute to an overwhelming glycaemic status.

World over, one of the major public health challenges of the 21st century is undisputedly T2DM. According to World Health Organisation (WHO) the epidemic of diabetes is strongly related to lifestyle and economic changes. Centre for Disease Control (CDC), Atlanta, US has projected data that shows approximately 200-300 million people worldwide will have developed T2DM by 2025 (CDC, NDFS, 2002) meaning an increase of nearly 6 million patients every year. Diabetes is the third leading cause among some tribal populations in South East Asian countries. Uncontrolled T2DM is associated with long-term microvascular and macrovascular complications with failure of vital organs, leading to nephropathy, atherosclerosis, retinopathy, renal failure, neuropathy etc. Insulin resistance and beta cell dysfunction are fundamental defects known to precede the onset of T2DM. The first signs of beta cell dysfunction can be detected 10-12 years prior to a full blown presentation of T2DM. Hence there is room for enormous opportunities and various parameters to target above defects in order to attempt to prevent and cure T2DM.

1.1. Current scenario in Malaysia

Diabetes is a growing health concern in Malaysia. The number of people with diabetes in Malaysia is increasing while complication rates and associated diseases amongst diabetics are significantly high. Occurrence of T2DM has also steadily increased over the years with an estimate of 0.65% in 1960, to 2% in 1982. In the National Health and Morbidity Survey (NHMS) carried out in 1986, the prevalence of diabetes mellitus was estimated to be 6.3%. In 1996, the Second National Health and Morbidity survey showed that the national prevalence of diabetes and impaired glucose tolerance in Malaysia were 8.3% and 4.8% respectively (Ooyub, 2004). In 1999, based on the prevalence among adults aged 30 years and above, there were 700,000 to 900,000 persons with diabetes. Currently there are around 1.2 million diabetics in Malaysia, with 98% of them diagnosed with T2DM. (PDM, 2007). This means there are approximately 8 diabetics in every 100 adults. The WHO has estimated that in 2030, Malaysia would have a total number of 2.48 million diabetics compared to 0.94 million in 2000 - a phenomenal 164% increase.

Statistics from the Ministry of Health records also shows that the number of admissions to Government Hospitals in Peninsular Malaysia for diabetes mellitus had increased (PDM, 2007). Admission to hospitals due to diabetes has increased from 19,629 cases in 1991 to 30,661 cases in 2001, which shows an increase of 56% over a span of 10 years. Mortality due to diabetes has also increased from 254 deaths in 1991 to 380 deaths in 2001 which is an increase of 50%.

Hospital-based data indicate complication rate as high as 50% (Mustaffa, 1983). Associated hypertension was seen in 10-20% of diabetics and,

hypercholesterolemia in 29% of patients. In one study, 38% were noted to have multiple complications, the commonest being hypertension and stroke, and gangrene with neuropathy. One reason for the high complication rates is poor glycaemic control. The Diabetes Care Data Collection Project (DCDCP) (Mustaffa, 1983) conducted in 1997 showed that more than half of diabetic patients were poorly controlled where 73% had HbA_{1c} more than 7.5% and 68% had fasting blood glucose more than 7.8 mmol/l. Few number of patients were monitored for renal function where only 30% were examined for proteinuria and only 1% were examined for microproteinuria. Eye examination was performed in only 3-20% while feet examination was conducted in 6-11% of cases. Glucose self-monitoring rate was less than 1%. The most prevalent chronic complications were neuropathy (58%) and retinopathy (53%).

Diabetes mellitus is strongly associated with obesity and the rise in the prevalence of diabetes is due to a rise in the prevalence of obesity. In the NHMS 2, 1996, the prevalence of obesity was 4.4% and of overweight was 16.6%. Amongst those with diabetes mellitus, 18.8% were either obese or overweight. In a study in Kelantan, 38.4% of diabetics were either obese or overweight compared to 24.1% in those with normal glucose tolerance (Mafauzy, 2006).

1.2. Pathophysiology of type 2 diabetes mellitus

T2DM affects more than 90% of all adults with diabetes, is a complex metabolic disease, characterised by elevated serum glucose levels (ADA, CPR, 2004). Fasting hyperglycaemia is caused by uncontrolled basal hepatic glucose output, an upshot of hepatic resistance to insulin action. Post-prandial hyperglycaemia results from abnormal insulin secretion by beta cells in response to

food, and a increase in hepatic glucose production, and defective glucose uptake by peripheral insulin-sensitive tissues. Chronic hyperglycaemia further impairs beta cell secretory kinetics and tissue sensitivity to insulin, known as glucotoxicity (Dailey, 2004). Thus, both impaired insulin action (insulin resistance) and impaired insulin secretion (insulin deficiency) are central to the pathogenesis of T2DM. Figure.1.1 represents pathogenesis of T2DM.

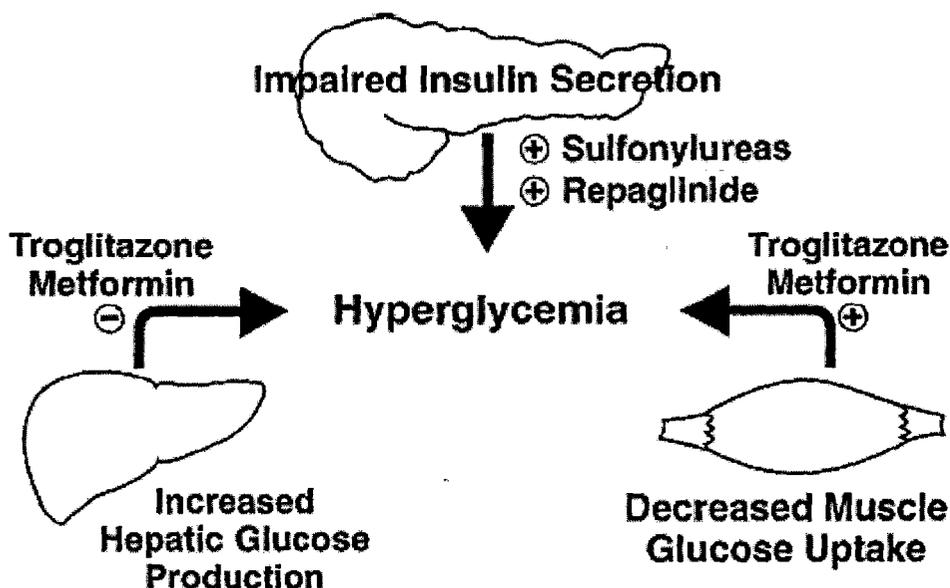


Figure 1.1 Pathogenesis of type 2 diabetes mellitus. Sites of action of oral agents are indicated. A negative sign indicates inhibition; a positive sign indicates stimulation. (DeFronzo, 1999)

On ingesting food, maintenance of normal glucose tolerance depends on the following three events in sync: 1) stimulation of insulin secretion; 2) insulin-mediated control of endogenous (primarily hepatic) glucose production by the resultant hyperinsulinemia; and 3) insulin-mediated stimulation of glucose uptake by peripheral tissues (primarily muscle). To a lesser extent, hyperglycemia can also independently suppress hepatic glucose production and enhance muscle glucose uptake. Accelerated gluconeogenesis is the major abnormality responsible for the increased rate of basal hepatic glucose production (Magnusson, 1992). The increased

rate of basal hepatic glucose production closely correlates with the increase in fasting serum glucose level. Since the fasting plasma glucose level is the major determinant of the mean day-long serum glucose level (clinically indicated by hemoglobin A_{1c} [HbA_{1c}] value), agents that reduce the elevated basal rate of hepatic glucose production will be especially effective in improving glycemic control. Muscle tissue in T2DM patients is resistant to insulin (DeFronzo, 1997; Bonadonna et al, 1996). Defects in insulin receptor function, insulin receptor-signal transduction pathway, glucose transport and phosphorylation, glycogen synthesis, and glucose oxidation all contribute to muscle insulin resistance (DeFronzo, 1997). In response to food, the ability of endogenously secreted insulin to boost muscle glucose uptake is markedly impaired (Mitrakou et al, 1990; Ferrannini et al, 1998). Hence muscle insulin resistance and impaired suppression of hepatic glucose production contribute equally to the excessive postprandial increase in the plasma glucose level (Ferrannini et al, 1998). So it is logical to expect that drugs causing an improvement in muscle insulin sensitivity will be effective in decreasing the excessive increase in plasma glucose level after carbohydrate ingestion.

Impaired insulin secretion also plays a major role in the pathogenesis of glucose intolerance in patients with T2DM (Polonsky, 1995). In the cascade of events, leading to full blown diabetes mellitus, all T2DM patients with elevated fasting serum glucose levels have a defect in insulin secretion (Polonsky, 1995). In diabetic patients with mild fasting hyperglycemia (glucose level, 7.8 mmol/l [140 mg/dl]), serum insulin levels during an oral glucose tolerance test or a mixed meal usually are elevated. As the fasting serum glucose level increases to more than 7.8 mmol/l, insulin secretion decreases progressively, and all diabetic patients with a

fasting serum glucose levels above 10.0-11.1 mmol/l (180-200 mg/dl) have a deficient serum insulin response. It follows, therefore, that drugs that improve insulin secretion will be effective in treating T2DM. In summary, patients with T2DM are characterized by defects in both insulin secretion and insulin action.

Maintaining serum glucose concentrations near the normal range by using insulin or oral antidiabetic agents has been demonstrated to prevent or delay the development of long term complications of T2DM (Lawrence, 2005). Weight control and physical activity are the predominant and effective non pharmacological ways to treat borderline type 2 diabetics. So it is common to switch over to pharmacological approaches at one point of time when non pharmacological interventions are ineffective in maintaining strict glycemic controls.

1.3. Clinical features of type 2 diabetes mellitus

A fasting serum glucose level above 7 mmol/l (126 mg/dl) on at least two occasions or random serum glucose of more than 11.1 mmol/l (200 mg/dl) with symptoms of polyuria and polydipsia are diagnostic indicators of T2DM (Nathan, 2002; Ahmann and Riddle, 2002). Subjects with impaired fasting serum glucose levels are often given an oral glucose tolerance test administered in the fasted state with consumption of a measured amount of a high glucose drink. Based on the excretion of glucose, with respect to time, individuals are grouped into three classes: normal, impaired, and diabetic.

A common management scheme may not be optimal for a disease with multifactorial causes. For borderline T2DM subjects, physicians usually recommend diet control and an increase in physical activity, but only ~20% of patients are

benefited by these interventions (Koro, 2004). The patients not helped by diet and exercise alone, or those who present with severe symptoms, are treated with one or more of 6 classes of drugs. These drugs target different pathways and organs: insulin secretion by the pancreas (sulfonylurea and meglitinides), glucose absorption by the intestines (alpha glucosidase inhibitors), glucose production in the liver (metformin), and insulin sensitivity in adipose and peripheral tissues (e.g., rosiglitazone and pioglitazone). A newly approved agonist of glucagon-like-peptide 1, exenatide, also acts in the pancreas to stimulate insulin production (Kwon, 2004), only when serum glucose levels are high. Approximately 50% of T2DM patients are prescribed oral medications only, about 11% prescribed combinations of oral agents with insulin, and the remainder takes no medications (20%) or insulin alone (16.4%) (Koro, 2004). Thus, current medical management of T2DM can be a lengthy trial and error method, involving significant amount of time and considerable expense.

1.4. Insulin resistance

Insulin resistance is said to be present when the biological effects of insulin are less than expected for both glucose disposal in skeletal muscle and suppression of endogenous glucose production primarily in the liver (Dinneen, 1992). In the fasting state, however, muscle accounts for only a small proportion of glucose disposal (less than 20%) whereas endogenous glucose production is responsible for all the glucose entering the plasma. Endogenous glucose production is accelerated in T2DM or impaired fasting glucose patients (Weyer, 1999; Meyer et al, 1998). Since this increase occurs in the presence of hyperinsulinaemia, at least in the early and intermediate disease stages, hepatic insulin resistance becomes the driving force of

When beta cells are no longer able to compensate for insulin resistance by adequately increasing insulin production, impaired glucose tolerance (IGT) appears (Kahn, 2003). This condition is characterized by an excessive blood glucose concentration in the postprandial phase, with fasting glucose being in the normal range (Kahn, 2003). Persistence of imbalance between caloric intake and expenditure eventually leads to overt diabetes, characterized by high glycemic status in any condition whether fasting or postprandial.

1.6. Pathogenesis of insulin resistance

The most important tissues involved in the pathogenesis of insulin resistance are muscle and adipose tissue. When caloric intake exceeds the energy expenditure, the substrate-induced increase in citric acid cycle activity generates an excess of mitochondrial NADH (mNADH) and reactive oxygen species (ROS) (Maddux, 2001). Hence cells may reduce the formation of ROS and/or enhance ROS removal. Prevention of ROS formation is accomplished by preventing the build-up of mNADH by inhibiting insulin stimulated nutrient uptake and preventing the entrance of energetic substrates (pyruvate, fatty acids) into the mitochondria.

Influx of substrates into the citric acid cycle generates mitochondrial acetyl-CoA and NADH (Maddux, 2001). Acetyl-CoA, derived either from glucose through pyruvate or from beta-oxidation of free fatty acids (FFA), combines with oxaloacetate to form citrate, which enters the citric acid cycle and is converted to isocitrate. NAD⁺-dependent isocitrate dehydrogenase generates NADH. When excessive NADH cannot be dissipated by oxidative phosphorylation (or other mechanisms), the mitochondrial proton gradient increases and single electrons are

transferred to oxygen, leading to the formation of free radicals, particularly superoxide anion (Maechler, 1999). The generation of excessive NADH may be prevented in several ways, one of which is the inhibition of FFA oxidation (Williamson and Cooper, 1980). An increase in intracellular FFA, in turn, leads to reduced GLUT4 translocation to the plasma membrane, resulting in resistance to insulin stimulated glucose uptake in muscle and adipose tissue (Tretter and Vizi, 2000; Rudich, 1998; Tailor, 2003). Here insulin resistance may be considered a compensatory mechanism that protects the cells against further insulin stimulated glucose and fatty acid uptake and therefore oxidative damage.

Initially, insulin resistance is compensated by hyperinsulinemia through which a normal glucose tolerance is preserved. Deterioration to IGT occur when insulin resistance increases further and/or the compensatory insulin secretory response decreases. An increase in insulin, FFA, and/or glucose levels can increase ROS production and oxidative stress, as well as activate stress-sensitive pathways. This, in turn, can worsen both insulin action and secretion, thereby accelerating the progression to overt type 2 diabetes.

1.7. Drug therapy in the treatment of type 2 diabetes mellitus and insulin resistance

Antidiabetics like sulphonylureas, biguanides, meglitinides, thiazolidinediones, and insulin have been the mainstays of treatment for type 2 diabetes mellitus and insulin resistance and are still in active use. Monotherapy for T2DM may fail with time as disease progresses this is when combination therapy is useful. Generally two different classes of drugs may be added with differing

mechanism of action thus promoting synergism with better control of symptoms. So coexisting disease conditions such as hypertension, high cholesterol levels, obesity, and potential for cardiovascular disease or complications must be taken into consideration before prescribing a combination therapy. The combination therapy most commonly prescribed are: sulphonyurea e.g. glyburide, glimepiride) with metformin, troglitazone with a sulphonylurea (glyburide) / insulin or pioglitazone with a sulphonylurea/ insulin, repaglinide with metformin, insulin (NPH) with a sulphonylurea.

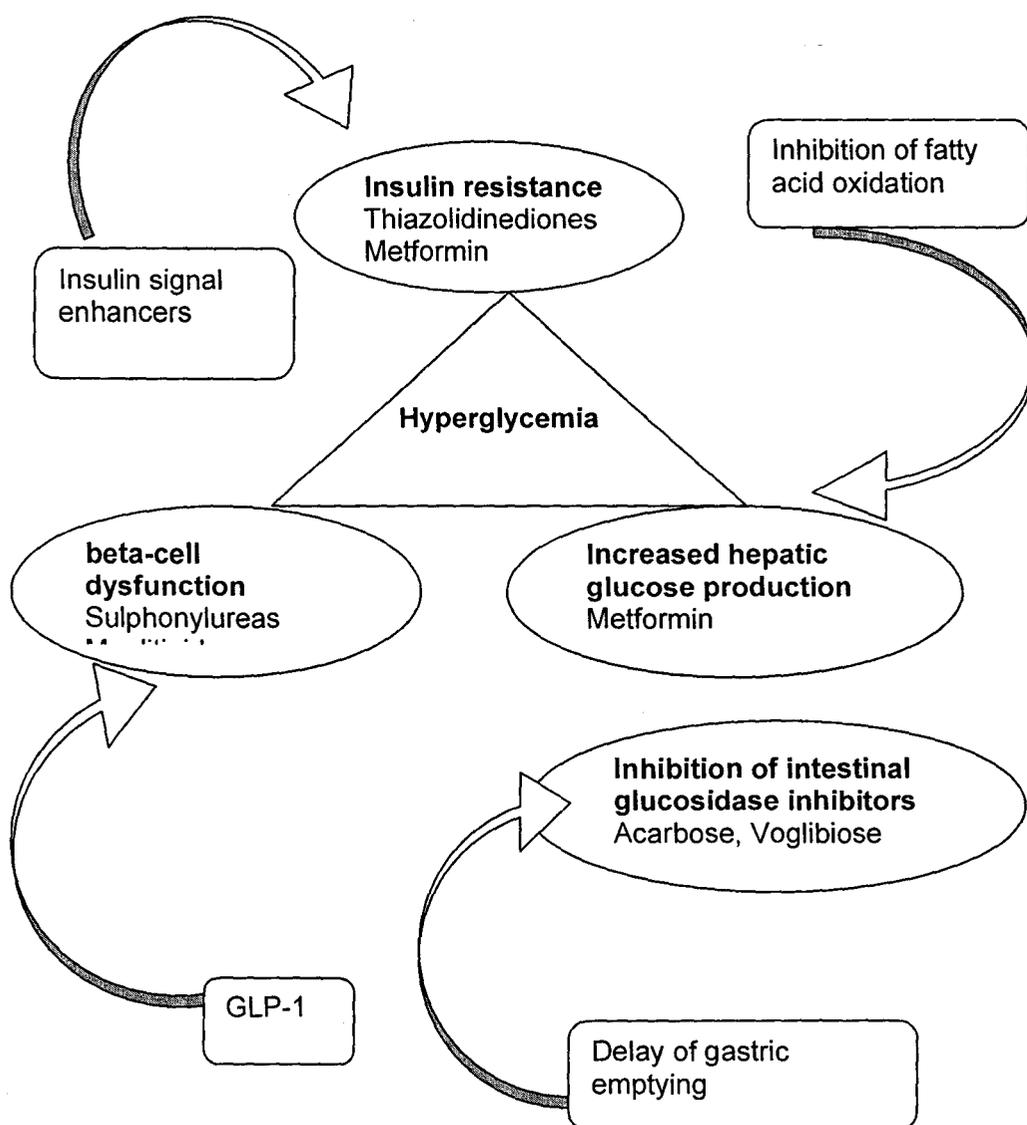


Figure 1.3 Mechanism of action of oral antidiabetic drugs. (Matthaei, 2000).

1.8. Problems of antidiabetic therapy

Though the oral antidiabetic therapy are found to be relatively safe and effective in type 2 diabetes and insulin resistance, each drug has its own range of side effects which may compromise the disease status or even worsen the condition in some cases (for example weight gain of sulphonylureas). Considering the fact that T2DM is a progressive disease with varied symptoms at each stage, treatment is also complicated, and usually the patients are prescribed with a combination of drugs once the disease attains a more chronic state. Some of the side effects which may offset the benefit from the therapy are as follows:

- Weight gain, hyperinsulinemia, and tolerance of sulphonylureas.
- Modest weight gain on treatment with meglitinides.
- Weight gain, edema, volume expansion on treatment with thiazolidinediones.
- Weight gain, patient non-compliance with insulin injections.

The last few years have been stagnant as far as new therapeutic options for oral agent for patients with T2DM are concerned and clearly there is a need for some newer specific and effective agents with action on multiple targets. Over the next several years, as the results of key clinical trials are revealed, the optimal therapeutic approach will likely be better defined, specifically regarding the best initial therapy for borderline and full blown T2DM patients. Such a choice may arise from studies exploring the cardiovascular and beta cell impact of various agents, particularly the insulin sensitizers (Kimmel and Inzucchi, 2005). Moreover new formulations of insulin such as for oral therapy, inhalational routes etc are finding success in research studies and also commercially, though limited. In the near future use of insulin in

various novel drug delivery systems will be a reality. Further, emerging concepts to be addressed may involve the progression to combination strategies in the pre-diabetic state, and liberal use of novel insulin formulations sooner in the disease course.

Obesity, the principle cause of type 2 diabetes, remains an important target for possible drug therapy. Available anti-obesity drugs have limited effectiveness on body weight; clearly, newer therapeutic options are needed (Kimmel and Inzucchi, 2005). Hence rimonabant-like drugs modulating the endogenous cannabinoid system and weight loss agents with more substantive effects on body weight will play an increasingly important role in the future therapy of obese T2DM patients. Availability of new information about safety, efficacy, and tolerability of newer agents from diabetic clinical trials that would significantly affect the way drugs are prescribed is eagerly awaited.

1.9. Current drugs in the pipeline

1.9.1 Exenatide

Exenatide (Byetta[®]), a synthetic version of the naturally occurring peptide exendin-4, has recently been approved by the US Food and Drug Administration (FDA) for the treatment of type 2 diabetes. It is a potent agonist of the glucagon-like peptide-1 (GLP-1) receptor, which acts primarily to reduce postprandial glucose excursions, with a lesser effect on fasting plasma glucose (FPG) levels (Drucker, 2001).

GLP-1 is an important incretin hormone whose secretion is reduced in individuals with type 2 diabetes. GLP-1 stimulates the secretion of insulin and

inhibits the secretion of glucagon in a glucose-dependent manner, dramatically lowering postprandial glucose levels (Drucker, 2001; Vilsboll, 2001), and regulates nutrient intake via effects on gastric emptying and feeding behaviour (Nauck et al, 1996; Turton et al, 1996). Therefore, a key benefit of GLP-1 mimetics may be improved glycaemic control with a decreased incidence of hypoglycemia relative to that observed with agents such as sulphonylureas and insulin, which act independently of glucose concentrations. GLP-1 is rapidly metabolised by dipeptidyl peptidase-IV (DPP-IV) immediately within few minutes of release thus limiting activity, necessitating the development of synthetic analogues or agonists. Exenatide is resistant to degradation by DPP-IV, with a half-life of 2-4 hrs, and is suited to twice daily injection. In clinical trials, it was administered at a dose of 10 µg twice a day for 82 weeks demonstrated a mean improvement in HbA_{1c} of 1.1% from baseline (Buse, 2004) with 4.5 kg mean weight reduction (Buse, 2004; DeFronzo, 2005; Kendall et al, 2005; Geelhoed-Duijvestijn, 2007).

The most frequent adverse event associated with exenatide therapy was nausea, which was generally mild or moderate in intensity and peaked during the initial weeks of dosing (weeks 0-8), before decreasing in incidence (Buse, 2004; DeFronzo, 2005; Kendall et al, 2005).

1.9.2. Pramlintide

Pramlintide (Symlin[®]) is an amylin analogue that is approved by the FDA for treatment of types 1 and 2 diabetes in patients who use mealtime insulin. Amylin is a peptide hormone that is co-secreted with insulin from the pancreatic beta cell and is therefore deficient in individuals with diabetes. It inhibits glucagon secretion, delays gastric emptying, and acts to enhance satiety. Natural amylin is liable to aggregate

and form amyloid fibres, which may play a part in beta cell destruction in type 2 diabetes, making it unsuitable for therapeutic use. Therefore, synthetic analogues have been developed that do not possess this characteristic (Schmitz, 2004). It is administered by subcutaneous injection prior to meals, in order to specifically reduce postprandial glucose levels.

Addition of pramlintide to insulin, metformin and sulphonylureas has demonstrated significantly reduced HbA_{1c} values from baseline compared with placebo. Additionally there was a slight decrease in body weight compared with an increase in body weight with placebo. The common side effects of pramlintide therapy are nausea and vomiting.

1.9.3. Rimonabant

FDA has approved rimonabant (Acomplia[®]) for obesity, metabolic disorders associated with diabetic conditions. It is first in class of novel cannabinoid receptor-1 antagonists (CB-1). The endocannabinoid system influences food intake by modulating the major 'reward' pathway in the mesolimbic dopaminergic system of the brain (Schlicker and Kathmann, 2001). Endogenous cannabinoids of the hippocampus and nucleus accumbens are involved in driving appetite for palatable food and therefore determine total energy intake and the severity of diet-induced obesity (Harrold, 2002). In clinical trials, rimonabant at a dose of 20 mg/day with sulphonylurea or metformin caused a decline in HbA_{1c} levels by 0.7% with effective reductions in weight reduction and waist circumference. Additionally HDL levels increased with simultaneous reduction in triglyceride levels (Scheen, 2005). The commonly encountered side effects at that dose were increased incidence of

psychiatric disturbances, such as depression and anxiety, compared with placebo (Despres, 2005; Vaan Gaal, 2005).

1.9.4. Vildagliptin

Vildagliptin (Galvus[®]) is a DPP-IV inhibitor, has completed Phase III clinical development and awaiting FDA approval for marketing. DPP-IV inhibitors act to extend the half-life of endogenous GLP-1 by blocking its degradation. Newer DPP-IV inhibitors in clinical development are administered orally, once daily, a potential advantage over the injectable GLP-1 agonists. In a clinical study vildagliptin was dosed at 50 mg once-daily in addition to metformin therapy in patients not achieving adequate glycaemic control. Patients receiving the combination showed significant reductions in both HbA_{1c} and fasting plasma glucose concentrations compared to placebo and vildagliptin did not significantly alter body weight (Ahren, 2004).

1.9.5. Ruboxistaurin

Ruboxistaurin (to be named Arxxant[®]) is a selective inhibitor of protein kinase C-beta (PKC-beta) that has been assessed in Phase III clinical studies for the treatment of diabetic retinopathy, nephropathy and neuropathy. Currently FDA has requested additional efficacy data to support the clinical evidence. Hyperglycaemia activates PKC-beta, and is associated with the development of microvascular complications in the retina, kidney and nervous system. These complex clinical sequelae are believed to be mediated by various mechanisms involving inflammatory mediators, endothelial activation and endothelial proliferation (Koya and King, 1998).

In a study of 252 patients with diabetic retinopathy, ruboxistaurin (8, 16 or 32 mg/day) did not prevent progression of the disease, but significantly delayed the occurrence of moderate visual loss (PKC-DRS, 2005).

1.9.6. Sitagliptin

Sitagliptin (Januvia[®]) orally-active inhibitor of the dipeptidyl peptidase-IV (DPP- IV) enzyme recently approved for marketing by FDA and is believed to exert its actions in patients with T2DM by slowing the inactivation of incretin hormones. Sitagliptin is indicated as an adjunct to diet and exercise to improve glycemic control in patients with T2DM. It is also indicated as combination therapy in patients with T2DM to improve glycemic control in combination with metformin or a PPAR- γ agonist (e.g., thiazolidinediones) when the single agent alone, with diet and exercise, does not provide adequate glycemic control (Sitagliptin, 2006).

Incretins, like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to food. These hormones are rapidly inactivated by DPP-IV enzyme. The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signalling pathways involving cyclic AMP. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, leading to reduced hepatic glucose production. By increasing and prolonging active incretin levels, sitagliptin increases insulin release and decreases glucagon levels in the circulation in a glucose-dependent manner.

In patients with type 2 diabetes, treatment with sitagliptin produced clinically significant improvements in HbA_{1c}, fasting plasma glucose (FPG) and 2-hour post-prandial glucose (PPG) compared to placebo. In a safety and efficacy study as monotherapy sitagliptin at 100 mg daily provided significant improvements in HbA_{1c}, FPG, and 2-hour PPG compared to placebo (Sitagliptin, 2006). The commonly encountered side effects were upper respiratory tract infection, stuffy or runny nose and sore throat, headache.

CHAPTER 2

Review of literature

2.0. *Andrographis paniculata*

2.1. Classification

Kingdom : Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Tubiflorae

Family : Acanthaceae

Genus: *Andrographis*

Species: *paniculata* Nees

2.2. Botanical Description

Andrographis paniculata (Burm.f.) Nees (Acanthaceae) is a traditional medicinal plant common in South East Asia and found from India to Indo-China. It is commonly called as king of bitter, kariyat, kalmegh, hempedu bumi and pokok cerita etc. It is an annual, erect and branched plant with lanceolate green leaves and attains heights of 60-70 cm. Since it is a traditional medicinal plant as usual it has various claims of uses and often with no literature supports, hence become difficult to verify. The leaves and aerial parts of the plant have been used to cure various kinds of ailments. Some of the uses are as follows: anti-pyretic, anti-periodic, anti-inflammatory, expectorant, depurative, sudoforic, anti-helminthic, digestive and stomachic. It is useful in hyperdyspsia, burning sensation, wounds, ulcers, chronic fever, malarial and intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, pruritis, intestinal worms dyspepsia, flatulence colic, diarrhoea, dysentery, and hemorrhoid



Figure 2.1 Leaves and aerial parts of *Andrographis paniculata*

Expressed juice of the leaves of *Andrographis paniculata* Nees alone or together with cardamom, cloves and cinnamon made into little globules that are prescribed as a domestic remedy for common conditions such as griping, irregular stools, loss of appetite, flatulence, and diarrhoea of children. Decoction or infusion of the leaves has been used in conditions of sluggish liver, neuralgia, certain forms of dyspepsia associated with gaseous distension of the bowels, in general debility, in convalescence after fevers and in advanced stages of dysentery. During epidemic of influenza, a tincture of the plant is highly efficacious in arresting the progress of the disease. The herb is also reported to possess astringent, anodyne, tonic and is helpful

in dysentery, cholera, diabetes, influenza, bronchitis, swellings, itches, piles and gonorrhoea. A decoction of the plant is used a blood-purifier. It is used as a cure for torpid liver and jaundice. A decoction or infusion of the leaves is useful in general debility and dyspepsia. The leaves and roots are also used as febrifuge, tonic, stomachic, cholagogue and anti-helminthic. It is used indigenously in medicine particularly as bitter tonic, curing fevers, dysentery and eliminating intestinal worms. It is also used as cholagogue. The plant is used to relieve griping, irregular stools and loss of appetite in case of infants and in debility and certain forms of dyspepsia. It is also reported to heal peptic ulcer.

2.3. Literature on *Andrographis paniculata*

One of the earliest known reports mentioning several traditional uses of *Andrographis paniculata* is that of Nadkarni and Nadkarni, 1976 which indicates several uses of *Andrographis paniculata*. These uses were practiced in ancient Ayurveda for the cure of several ailments notable among them being anti-pruretic, anti-inflammatory, anti-diarrheal and as laxative, expectorant, depurative etc. It is also used in diseases of infectious origin like malaria and dysentery.

Ancient Chinese physicians used it to treat inflammatory conditions, fever, cold, laryngitis and has been described as a cold property herb to get rid of body heat and dispose toxins from body (Deng, 1982). Dutta and Sukul, 1982 reported the anti-inflammatory activity of deoxyandrographolide, andrographolide, and neoandrographolide from *Andrographis paniculata* leaf powder in rats.

According to the study conducted by Shahid, 1987 pretreatment with *Andrographis paniculata* aqueous extract demonstrated significant hepatoprotective effect in the *in vivo* study as evidenced by the subsequent histopathology and liver enzyme levels. Further hepatoprotective study of andrographolide (the major active diterpenoid lactone of the plant *Andrographis paniculata*) was studied on acute hepatitis by Handa and Sharma, (1990a) induced in rats by a single dose of galactosamine (800 mg/kg, ip) and paracetamol (3 g/kg, po). Results indicated pre-treatment and post-treatment of rats at different time intervals with different doses of andrographolide in the two experimental models of hepatotoxicity lead to complete normalisation of toxin-induced increase in the levels of all the five biochemical parameters and significantly ameliorated toxin-induced histopathological changes in the livers of experimental rats. The results confirmed the *in vivo* hepatoprotective effect of andrographolide against galactosamine or paracetamol-induced hepatotoxicity in rats.

Another anti-hepatotoxic study of andrographolide (100 mg/kg, ip) was carried out by Handa and Sharma, (1990b) comparing the activity of 861.33 mg/kg, ip, of the methanolic extract (equivalent to 100 mg/kg of andrographolide) and 761.33 mg/kg, ip, of the andrographolide-free methanolic extract (equivalent to 861.33 mg/kg of the methanolic extract) of the plant, in CCl₄-induced liver damage in rats. Biochemical parameters like serum transaminases- GOT and GPT, serum alkaline phosphatase, serum bilirubin and hepatic triglycerides were estimated to assess the liver function. Overall inhibition of CCl₄-induced increase in the five biochemical parameters was found to be 48.6% (andrographolide), 32% (methanolic extract) and 15% (andrographolide-free methanolic extract) and 15%

(andrographolide free methanolic extracts). Andrographolide (100 mg/kg, ip) was also found to normalize completely the CCl₄-induced increase in the pentobarbitone induced sleep time of mice. The results suggest that andrographolide is the major active anti-hepatotoxic principle present in *Andrographis paniculata*.

Alcoholic extract of the leaves of *Andrographis paniculata* obtained by cold maceration at a dose of 300 mg/kg was selected to study hepatoprotective action against CCl₄-induced liver damage. The extract was found to be effective in preventing liver damage which was evident by morphological, biochemical and functional parameters (Rana and Avadhoot, 1991).

Study conducted by Visen, 1991 showed that andrographolide produced a dose (1.5-12 mg/kg, 7 days once daily) dependent choleric activity in conscious rat as well as anaesthetized guinea pig. It also showed a significant anti-cholestatic effect (40-100%) against galactosamine induced hepatic damage. The compound showed significant anticholestatic effect (40-100%) against galactosamine induced hepatic damage. It also showed a significant hepatoprotective activity (20-100%) by increasing the viability of hepatocytes as tested by trypan blue exclusion and oxygen uptake tests. Andrographolide reversed the altered values of GOT, GPT and alkaline phosphatase in hepatocytes and serum. Andrographolide was found to be more potent than silymarin, a known hepatoprotective drug.

The effect of crude ethanol extract of *Andrographis paniculata* was studied on experimental parasitaemia. A four-day suppressive test against *Plasmodium berghei* NK 65 in *Mastomys natalensis* (Misra, 1992) was carried out. The crude

ethanol extract and the fractions reduced the level of parasitaemic load, but not in a dose-dependent manner. Chemoprophylactic activity of neoandrographolide was tested using different protocols. Pretreatment for 15 days with neoandrographolide before infection suppressed the parasitaemia.

In another study on the alcohol extract of *Andrographis paniculata* (25 mg/kg) and two of its constituent diterpenes andrographolide and neoandrographolide (6 mg/kg/day for two weeks) showed significant antihepatotoxic action in *Plasmodium berghei* K173-induced hepatic damage in *Mastomys natalensis* (Chander, 1995). The increased levels of serum lipoprotein-X, alkaline phosphatase, GOT, GPT and bilirubin were markedly reduced by *Andrographis paniculata* and its diterpenes. In the liver, the extract and its constituents decreased the levels of lipid peroxidation products and facilitated the recovery of superoxide dismutase and glycogen. The protective effects of andrographolide were comparable to those of neoandrographolide.

Oral administration of andrographolide isolated from *Andrographis paniculata* leaves, (30, 100, and 300 mg/kg) was studied for its analgesic, anti-pyretic and anti-ulcerogenic activities (Madav, 1995). Andrographolide did not show any analgesic activity in hot plate test in mice while it showed significant analgesic activity in acetic acid-induced writhing in mice and Randall Selitto's test in rats at 300 mg/kg dose. Andrographolide (100 and 300 mg/kg, po) produced significant ($p < 0.05$) anti-pyretic effect after 3 hrs of administration in Brewer's yeast-induced pyrexia in rats. Andrographolide also exhibited significant anti-ulcerogenic activity at 100 and 300 mg/kg doses in aspirin- induced gastric-ulceration experiment in rats.