INTRODUCTION

Mulberries *(Morus alba L., Moraceae)* have been widely used in far east for several applications including treatment of DM ***(Kang et al., 2006).*** It contains various nutritional components such as flavonoids, polyphenols and especially 1- DNJ a potent glucosidase inhibitor, which shows hypoglycemic ***(Park et al., 2005)*** hypolipidemic and antiatherogenic effects in certain animal models ***(Enkhmaa et al., 2005).***

Chiasson et al. (2002) and ***Kawamori et al. (2009)*** study have demonstrated that α-glucosidase inhibitors reduced the progression from impaired glucose tolerance to type 2 DM therefore, considerable attention has been paid to α-glucosidase inhibitors as preventive and therapeutic agents for type 2 DM and its complications. MBL have been used as a Chinese herbal tea, especially for treatment of DM. In the modern era, health benefits from mulberry products have been verified scientifically, with MBL shown to have the most potent α-glucosidase inhibitory effect thus playing an important role in treatment of DM.

Epidemiological evidence indicated that postprandial hyperglycemia is an independent risk factor for cardiovascular disease ***Bonora and Muggeo (2001). Aramwit et al. (2011)*** research have also found that MBL was effective in reducing lipid profile in mild hyperlipidemic patients. Also ***Harauma et al. (2007)*** has found that MBL powder can prevent atherosclerosis in apolipoprotein E-deficient mice.

Aim of the Study

The present study was conducted to study and evaluate the therapeutic efficacy of Egyptian MBL in diabetic animal model.

MULBERRY

Morus alba of the family (*Moraceae*), sometimes referred to as Mori Albae or White Mulberry, is a medicinal fruit with roots and bark also having some medicinal usage with traditional Chinese Medicine using white mulberry for the purposes of antiphlogistic, diuretic, antitussive, expectorant, anti headache and antipyretic (Table 1).

Table (1): Taxonomy

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Urticales
Family	Moraceae
Genus	Morus
Species	Morus alba

Two varieties of **MBL** are recognized (Figure 1).

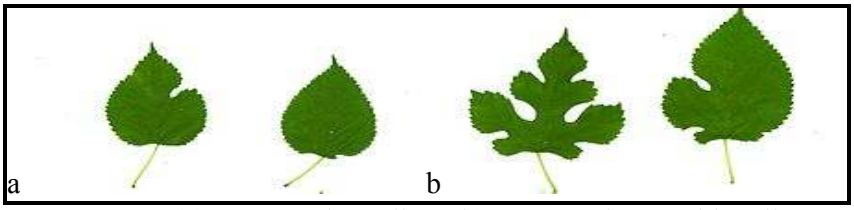

Fig. (1): (a) MBL variety alba. (b) MBL variety multicaulis ***(Zhengyi et al., 2013).***

Medical uses of MBL

Mulberry leaves contain a powerhouse of highly active ingredients and beneficial minerals, such as beta-carotene, GABA-1, amino acids,

carotenoids, flavonoids, chlorophyll, vitamin C, B1, B2, B6 and A. They are rich in azasugars fibers such as DNJ, fagomine, N-methyl-DNJ, and 2-O-R-D-galactopyranosyl the ingredient of particular interest is 1-DNJ which can only be found in MBL ***(Inoue et al., 1967)***.

Azasugars are alkaloids that mimic the structures of monosaccharides they have α-glucosidase inhibitory properties because of their ability to competitively bind to the active sites of α- glucosidase enzyme by mimicking the corresponding natural substrates. DNJ is the dominant alkaloid, accounting for 50% of mulberry azasugars ***(Asano et al., 2001)***, also naturally occurring DNJ may be isolated from the roots of mulberry trees and called moranoline ***(Yagi et al., 1976)***.

The histopathologic studies undertaken on the islets demonstrated the NH recovery of damaged islets and an improvement in the number of β cells after treatment with the plant extract. It can thus be assumed that MBL extract has a therapeutic effect that alleviates DM ***(Jamshid et al., 2012).***

Mulberry leaves also contain kuwanon C, mulberrofuran G and albanol B, all showed strong antibacterial activity with minimum inhibitory concentrations ranging from 5 to 30 mg/ml ***(Sohn et al., 2004)***. Various fractions of mulberry such as chloroform extract had strong antimicrobial activities against bacilllus subtilis and fractions extracted with acetic acid against on 1,1-diphenyl-2-picrylhydrazyl radical ***(Chung et al., 2003)***.

Dietary consumption of MBL and major flavonol glycoside, quercetin 3-(6-malonylglucoside) are associated with reduced atherosclerotic lesion area through enhancement of LDL resistance to oxidative modification in LDL receptor deficient mice ***(Enkhmaa et al., 2005)***. Butanolic extract of MBL scavenged the 1,1-diphenyl-2-picrylhydrazyl radical and inhibited the oxidative modification of rabbit and human LDL ***(Katsube et al., 2006)***. Quercetin and its conjugates are major representatives of the flavonol group of mulberry ***(Doi et al., 2000)***. These flavonoids exhibited strong inhibitory effects on oxidative modification of human LDL in vitro ***(Mezzetti et al., 2000; Day and Williamson, 2001; Naderi et al., 2003)***.

Methanolic extract from MBL and its subfractions (chloroform, butanol, and aqueous fractions) inhibited NO production and significantly decreased the production of TNF in macrophages ***(Choi and Hwang, 2005)***

Utilization of MBL reduces the risk of Alzheimer's disease. MBL extract provided a viable treatment for Alzheimer's disease through the inhibition of amyloid beta-peptidefibril formation and attenuation of amyloid β-peptide induced neurotoxicity ***(Niidome et al., 2007).***

Extracts from MBL exhibited high inhibition on the 3,4-dihydroxyphenylalanine oxidase activity and antityrosinase activity ***(Lee et al., 2002)***. Oxidative stress caused by free radicals was proposed for hyperpigmentation. According to ***Hogade et al. (2010)*** the alcoholic and aqueous extract of MBL possessed hepatoprotective activity.

Kim et al. (2012) research have shown that the methanolic extract of MBL may possess anti-dopaminergic effects, as it has succeded to augment haloperidol and metoclopramide induced catalepsy in mice when MBL is ingested at 50-200mg/kg and has also prolonged phenobartitol induced sleep time while reducing amphetamine-induced fights in mic***e (Yadav and Nade, 2008)***. MBL extract assisted in restoration of the vascular reactivity of diabetic rats. Free radical-induced vascular dysfunction plays a key role in the pathogenesis of vascular disease found in chronic diabetic patients. An ethanolic extract of MBL had antihyperglycemic, antioxidant and antiglycation effects in chronic diabetic rats, which may suggest its use as food supplement for diabetics ***(Naowaboot, 2009).***

According to ***Bahrani et al. (2010)*** MBL extract has got effect on humoral immunity in the indirect haemagglutination test, serum immunoglobulin levels and mice lethality test and it also has effect on the cell mediated immunity as it showed significant increase in the neutrophil adhesion, carbon clearance and a reduction in cyclophosphamide induced neutropenia protective effects.

Vikram et al. (2013) have proven that MBL extract partially inhibited the pro-coagulant activity and completely abolished the stimulation of 5-adenosine monophosphate-activated. MBL extract increases the activity of both adenosine monophosphate-activated _1 and _2 in skeletal muscle, and that this increase is associated with insulin-independent glucose transport without change in the energy status of the muscle. It has been proposed that MBL fosters a metabolic milieu that reduces the risk of type 2 DM at least in part by activating skeletal muscle.

In traditional Chinese medicine, the fruit is used to treat prematurely grey hair, to "tonify" the blood and to promote urination, and treat constipation and DM. The bark is used to treat cough, wheezing and edema. The fruit are also eaten, often dried or made into wine ***(Bean, 1978).***

Also mulberry fruits has been used effectively in natural medicine for the treatment of sore throat, fever, hypertension and anemia ***(Voutilainen et al., 2006)***. Moreover, mulberry fruit is used to protect against liver and kidney damage, strengthen the joints, improve eyesight and have anti-aging effects ***(Record et al., 2001).*** Anthocyanins and water extracts from mulberry fruit can scavenge free radicals, inhibit LDL oxidation and have beneficial effects on blood lipid and atherosclerosis ***(Ning et al., 2005). Yang et al. (2010)*** have proven hypolipidemic and antioxidant effects from freeze-dried powder of mulberry fruit.

DIABETES MELLITUS

Diabetes mellitus is defined as a disorder of carbohydrate metabolism caused by absence or deficiency of insulin, insulin resistance, or both, ultimately leading to hyperglycemia. DM is typically classified into two main subtypes: type-I or insulin-dependent diabetes and type-II or non-insulin-dependent diabetes. A more accurate way to differentiate the two would be to classify the insulin dependent diabetic as ketoacidosis-prone, and the non-insulin-dependent diabetic as ketoacidosis-resistant. Type-I and II would be differentiated on immunological-etiological grounds with type-I referring to an immune-mediated condition, whereas type-II is non-immune-mediated ***(Foster et al., 1988).***

This classification would result in the potential for three groups a type-I insulin-dependent group, a type-I non-insulin-dependent group, and a type-II non-insulin-dependent group. The type-I insulin-dependent group would encompass those non-obese diabetics for whom insulin is not yet required to prevent ketoacidosis but for whom islet cell antibodies are present in the blood. This group can be considered to have type-I insulin-dependent diabetes in evolution. The destruction of islet cells occurs gradually over time, so there is a delay in reaching the insulin dependency stage ***(Kathleen and Head, 1997).***

Pathogenesis of Type-I Diabetes mellitus

It is believed that type-I DM has a genetic component which must be present for susceptibility to occur. Although the exact mechanism is

unclear, transmission is believed to be autosomal dominant, recessive or mixed although no mechanism has been proven. If a first-degree relative has insulin dependent DM, the child has a 5-10% chance of developing type-I DM ***(Foster et al., 1988)***.

It is believed that the susceptibility gene resides on the sixth chromosome, with the major alleles conferring risk being HLA-DR3, HLA-Dw3, HLA-DR4, HLA-B8 and HLA-B15. Secondly, an environmental insult, such as a virus, exposure to an allergen, or both is believed to initiate the process in genetically susceptible individuals. This external influence precipitates an inflammatory response in the pancreas known as insulitis. Activated T lymphocytes infiltrate the islet cells in the pancreas. macrophages and T-cells appear to be implicated in beta-cell destruction via localized release of cytokines ***(Gale, 1996)***.

Cytotoxic amounts of NO and reactive oxygen intermediates are also released contributing to free radical damage to the beta cells. The initial steps in free-radical induced islet cell death involve breaks in DNA strands and the activation of the enzyme poly(ADP-ribose)polymerase is involved in DNA repair and consumes large amounts of NAD+ in the process. The depletion of intracellular NAD+ pools leads to islet cell death ***(Heller et al., 1997)***.

Schmernthaner (1995) have proven that the inflammatory response is autoimmune mediated and takes place on the surface of the insulin-producing β cells such that these cells are no longer recognized by the immune system. Antibodies against the β cells are produced, resulting in their destruction and the clinical appearance of DM. This destruction is thought to occur slowly over the course of several years in many cases.

Some viruses seem to attack and destroy the beta cells directly rather than initiating an autoimmune reaction (Figure 2).

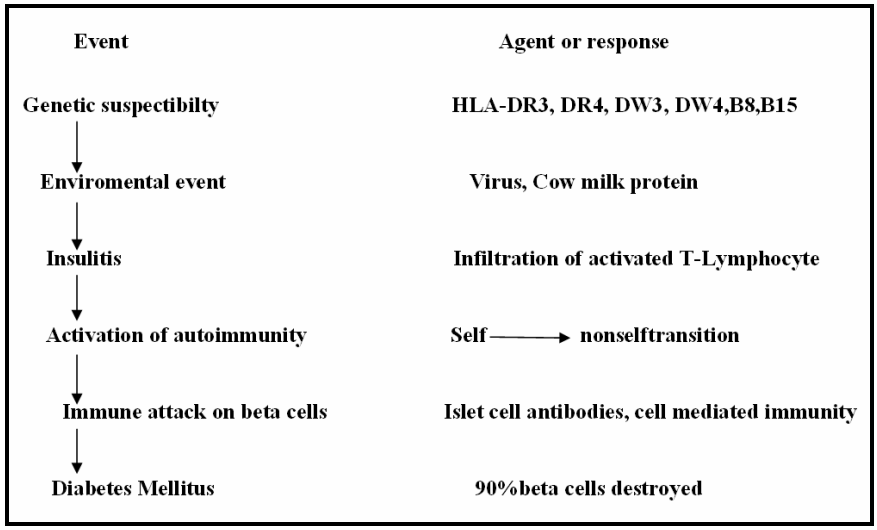

Fig. (2): The Pathogenesis of Type 1 DM ***(Kathleen and Head, 1997).***

Pathophysiology of Type 2 Diabetes Mellitus

In type 2 Diabetes mellitus, there is a steady decline of β-cells that add to the process of elevated blood sugars essentially, if someone is resistant to insulin, the body can, to some degree, increase production of insulin and overcome the level of resistance. After time, if production decreases and insulin cannot be released vigorously, insulin resistance develops ***(Brad et al., 2011).***

Insulin resistance can be defined as the inability of insulin to produce its usual biological actions at circulating concentrations that are effective in normal subjects. Insulin resistance in the context of glucose metabolism leads to impaired suppression of endogenous glucose production, under basal conditions as well as after eating, when the

physiological rise in insulin in response to glucose entry from the gut normally shuts down glucose production by the liver, and to reduced peripheral uptake of glucose. These alterations result in hyperglycemia and a compensatory increase in insulin secretion ***(John et al., 2011).***

Resistance to the ability of insulin may suppress VLDL production from the liver increases circulating serum TAG, which, in turn, leads to a decrease HDL and formation of atherogenic, small, dense, LDL particles. Resistance in adipose tissue increases the flux of non-esterified fatty acids both to the liver and skeletal muscle, and impairs the action of insulin on glucose metabolism in these tissues Resistance to other actions of insulin, such as its vasodilator and anti platelet aggregation effects, also characterize insulin resistance in patients with type 2 DM ***(Reaven, 1988)*** (Figure 3).

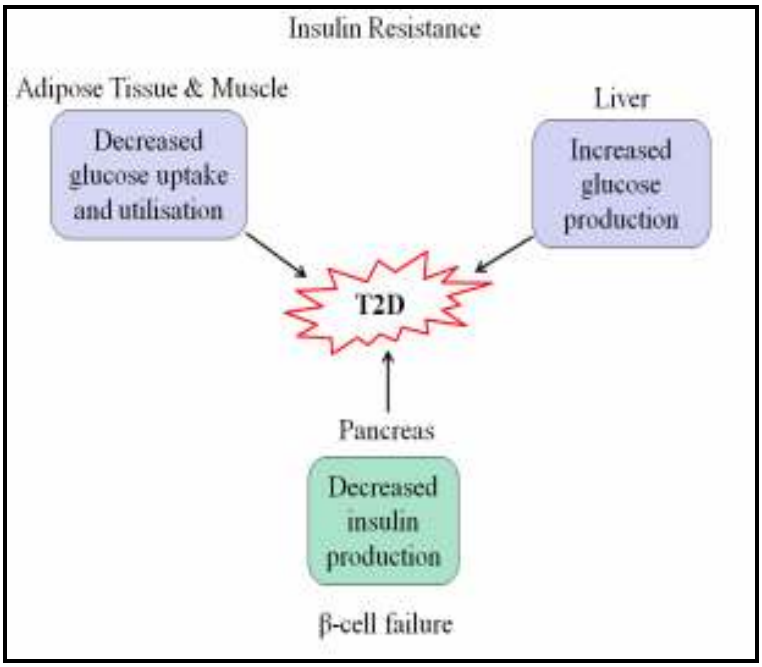

Fig. (3): Pathophysiology of Type2 DM ***(Brad et al., 2011)***.

Pathophysiology of Diabetic Dyslipedemia

Increased hepatic production and/or retarded clearance from plasma of large VLDL also results in increased production of precursors of small dense LDL particles and many LDL subspecies, which differ in their metabolic behavior and pathological roles**.** Plasma VLDL levels correlate with increased density and decreased size of LDL ***(McNamara et al., 1987; McNamara et al., 1992).***

LDL size and density are inversely related to plama levels of HDL, especially the small dense LDL particles appear to arise from the intravascular processing of specific larger VLDL precursors through a series of steps, including lipolysis. Also TAGs enrichment of the lipolytic products through the action of cholesteryl ester transfer protein together with hydrolysis of TAG and phospholipids by hepatic lipase, leads to increased production of small dense LDL particles ***(Krauss, 1998).*** Plasma residence time of these LDL particles may be prolonged because of their relatively reduced affinity for LDL receptors ***(Berneis and Krauss, 2002)***. The reductions in HDL particles associated with type 2 DM and insulin resistance are multifactorial, but a major factor appears to be increased transfer of cholesterol from HDL to TAG rich lipoproteins, with reciprocal transfer of TAG to HDL ***(Hopkins and Barter, 1986)*** (Figure 4).

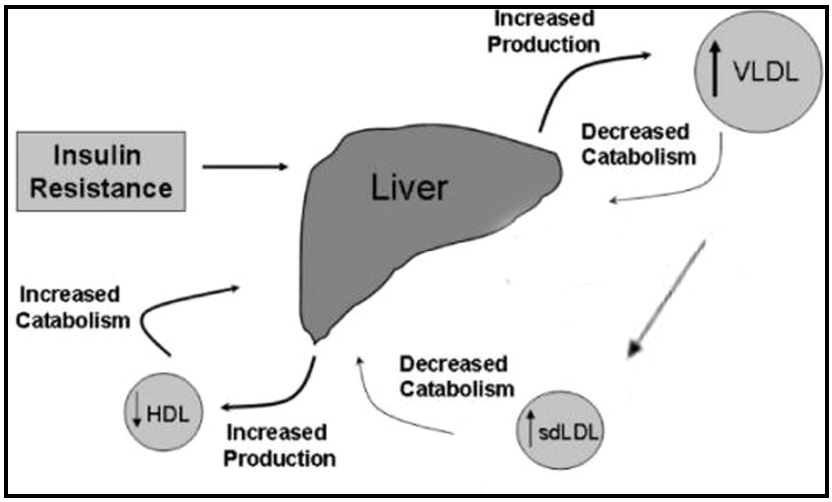

Fig. (4): Pathophsiology of Diabetic Dyslipidemia
(Krishaswami and Vijayaraghavan, 2010).

Diabetic Complications

Diabetes mellitus is a major source of morbidity, mortality to the society. People with DM showed the risk of the development of acute metabolic complications such as diabetic ketoacidosis, hyperglycemic, hyperosmolar non ketoticcoma and hypoglycaemia ***(English and Williams, 2004)***.

Diabetics are also at risk of experiencing chronic complications such as coronary heart diseases, retinopathy, nephropathy and neuropathy, and foot ulceration ***(Tiwari et al., 2013).*** It has been observed that insulin secretion declines with advancing age, and this decline may be accelerated genetically. Insulin resistance typically precedes the onset of type 2 DM and is commonly accompanied by other cardiovascular risk factors: dyslipidemia, hypertension, and prothrombotic factor**s** ***(Mezzetti et al., 2000)***. It has been shown that low carbohydrate ketogenic diet is effective

in the amelioration of many of the deleterious consequences of DM ***(Al-Khalifa et al., 2011)*** (Figure 5).

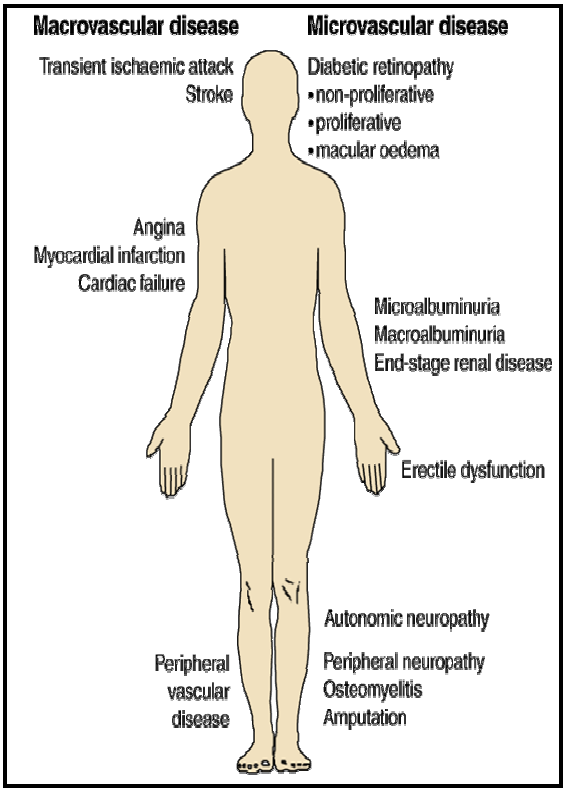

Fig. (5): Major Diabetic Complications ***(Katherine etal., 2010)***.

Role of Oxidative Stress in Diabetic Complications

The balance between the rate of free radical generation and elimination is important. Excess cellular radical generation can be harmful ***(Rice et al., 1997).*** However, if there is a significant increase in radical generation, or a decrease in radical elimination from the cell, oxidative cellular stress ensues ***(Valko et al., 2007)***. There is convincing experimental and clinical evidence that the generation of ROS increases in

both types of DM and its onset is closely associated with oxidative stress ***(Johansen et al., 2005).*** Oxidative stress results from increased ROS and/or reactive nitrogen species ***(Joseph et al., 2003)***. Examples of ROS include charged species such as superoxide and the hydroxyl radical, and uncharged species such as hydrogen peroxide and singlet oxygen ***(Rosen et al., 2001).***

The possible sources of oxidative stress in DM might include auto-oxidation of glucose, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants, such as GSH and vitamin E, and impaired activities of antioxidant defence enzymes such as SOD and CAT ***(Haskins et al., 2003).*** ROS generated by high glucose is causally linked to elevated glucose and other metabolic abnormalities important to the development of diabetic complications. Evidence has established the role of free radicals and oxidative stress in the pathogenesis and development of complications from DM ***(Lobo et al., 2010)*** including retinopathy, nephropathy, neuropathy, and accelerated coronary artery disease ***(Phillips et al., 2004).***

The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has direct effect on onset of type 2 DM via insulin resistance. The underlying mechanisms in the onset of DM are complex because hyperglycemia could also be due to the cause-effect relationship of increased oxidative stress. Biomarkers of increased oxidative stress, as measured by indices of lipid peroxidation and protein oxidation, increase in both Type 1 DM, and Type 2 DM ***(Cederberg et al., 2001).***