# 5. DISCUSSION

Type 2 diabetes mellitus is a metabolic disorder of fuel homeostasis characterized by hyperglycemia and altered lipid metabolism caused by islet β cells being unable to secrete adequate insulin in response to varying degrees of over nutrition, inactivity, consequential overweight or obesity, and insulin resistance. The burden of this disorder is enormous, owing to its rapidly increasing global prevalence, the devastating damage it can do to many organs of the body, and the direct and indirect costs. The estimated worldwide prevalence of diabetes among adults was 285 million (6.4%) in 2010, and this value is predicted to rise to around 439 million (7.7%) by 2030.[17] Surprisingly, this estimates of the global prevalence of diabetes indicate that, Egypt will be ranked as the 10$^{th}$ country worldwide which have the highest number of people with diabetes (6.7 millions).[18] The pandemic of type 2 diabetes, along with its high human and economic costs, is showing no signs of abatement and, therefore, new approaches are urgently needed to prevent, slow the progression, and limit the consequences of this disease.[26]

The treatments of diabetes include diet, exercise, use of oral hypoglycemic agents and insulin is the primary forms of treatment for diabetes. Currently available synthetic anti-diabetic agents besides being expensive produce serious side effects. Apart from currently available therapeutic options, traditional medicines or complementary and alternative medicines are a fruitful source of future drugs to counteract insulin resistance, consistent with a resurgence of interest in drug discovery from natural products.[142] A major advantage of traditional medicine is that they have been used to treat human diseases for many years and so there is considerable knowledge concerning in vivo efficacy and safety, two of the confounding problems facing other new chemical entities. However, in most cases there is little rigorous scientific evidence proving their efficacy and the mode of action is generally not known. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy. A hypoglycemic action from some treatments has been confirmed in animal models and non-insulin-dependent diabetic patients, and various hypoglycemic compounds have been identified. A botanical substitute for insulin seems unlikely, but traditional treatments may provide valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts.[143] The efficacy of hypoglycemic herbs is achieved by increasing insulin secretion, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose absorption from intestine and inhibiting glucose production from hepatocytes. It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and alternative medicine.[144]

Bitter melon (Momordica charantia) is a popular fruit used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. Abundant pre-clinical studies have documented the anti-diabetic and hypoglycemic effects of bitter melon through various postulated mechanisms; however the precise mode of action is still unclear.[118] This thesis aimed to evaluate the hypoglycemic activity and the probable underlying mechanisms of action of Bitter melon extract in diabetic rats by studying the changes in insulin signaling pathway in different peripheral tissues.

There is little doubt that some animal models of diabetes have provided an invaluable insight into the pathogenesis of the human disease. Patients have directly benefited from the use of animals in the discovery of insulin and the assessment of other treatments. Syndromes resembling human diabetes occur spontaneously in some animal species. Alternatively, they can also be induced by treating animals with drugs or viruses, excising their pancreases, or manipulating their diet. Of course, none of the known animal models can be taken to reproduce human diabetes, but they are believed to illustrate various types of etiological and pathogenic mechanisms and to evaluate the efficacy and toxicity of drugs that could also operate in humans. Among these models, diabetes induced in rats by neonatal streptozotocin (STZ) administration (so-called "n-STZ models") has been recognized as an adequate tool to study the long-term consequences of a gradually reduced beta-cell mass and impaired insulin action.[145] The diabetic syndrome in this model is generated by injecting wistar rats on the $5^{th}$ day of their birth (n5=birth) intra-peritoneally with 100 mg/kg of STZ. This model has many advantages over other models of diabetes and is considered to be one of the suitable experimental animal models of type 2 diabetes mellitus.[129]

The results indicated that, the n-STZ diabetic rat model showed the typical manifestations of type 2 diabetes mellitus (T2DM) including increased weight gain, hyperglycemia, decreased insulin level and elevated HOMA-insulin resistance index compared to control rats. Also in the diabetic rats, the impaired glucose homeostasis is associated with disturbed lipid metabolism as indicated by elevated triglycerides, total cholesterol and LDL-cholesterol and decline in HDL-cholesterol.

The treatment of the diabetic rats with serial doses of ethanolic extract of bitter melon showed a dose dependent decrease in body weight and increase in fasting insulin level while the fasting blood sugar and HOMA-insulin resistance index showed dose-dependent decline with the doses from 100 to 400 mg/kg with the best results obtained with 400 mg/kg dose, the highest dose (600 mg/kg) showed significant elevated values compared to untreated diabetic rats. When comparing the efficacy of bitter melon with conventional drugs like sulfonylurea (glibenclamide) we found that bitter melon extract at dose of 400 mg/kg showed comparable improvements in glucose homeostasis parameters with glibenclamide. These findings are in line with the reported similar hypoglycemic effects of bitter melon and glibenclamide in alloxan treated diabetic rats.[123]

There are a compiling number of studies that support the observed hypoglycemic effects of bitter melon. In line with our results the ethanolic extract of bitter melon (250 mg/kg dose) significantly lowered blood sugar in fasted as well as glucose loaded non-diabetic rats.[146,147] The ethanolic extract of (200 mg/kg) showed an anti-hyperglycemic as well as hypoglycemic effect in normal and STZ-diabetic rats as evident by 23% and 27% decrease in blood sugar, respectively.[148] Mohammady et al.,(2012) showed that treatment of diabetic rats with bitter melon produced a significant increase in fasting serum insulin as compared to the diabetic untreated group. The same finding is reported in many studies.[149,150] On contrary, Toshihiro et al., (2001) and Subratty et al., (2005) reported that treatment of diabetic rats with bitter melon decreased serum insulin. This discrepancy may be related to the diabetic model used.[151,152] In accordance with our data, Ayoub et al., (2013) documented that bitter melon was as effective as glibenclamide in alleviating STZ induced diabetic effects and has a dose dependent antidiabetic effects.[153]

Another study reported that bitter melon supplementation inhibit adiposity in rats feed high fat diet.[154] Also, it was reported that bitter melon could be more effective in the management of diabetes and its related complications as compared to rosiglitazone.[155]

Bitter melon is a powerful nutrient-dense plant composed of a complex array of beneficial compounds. These include bioactive chemicals, vitamins, minerals and antioxidants which all contribute to its remarkable versatility in treating a wide range of illnesses. The fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B1, B2 and B3, as well as vitamin B9 (folate).[156] The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber. Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates, all of which confer a bitter taste.[157] In the present study and due to several advantages of ethanol over acetone, such as being naturally derived, having low cost, and being more widely used as processing solvent in food and pharmaceutical industries, it was chosen as extraction solvent for subsequent investigations of bitter melon fruits.

Due to its high constituents of active ingredients there are multiple pathways through which bitter melon could induce anti-diabetic and hypoglycemic effect in the diabetic rats. These pathways include; inhibition of glucose absorption, induction of insulin secretion and insulin signaling and sensitivity, and induction of glucose disposal.

Bitter melon may act through inhibition of glucose absorption through inhibition of amylase and α-glucosidase enzyme activities.[158] $Na^+$- and $K^+$-dependent glucose absorption in the brush border membrane vesicles of the jejunum was found to be inhibited by bitter melon extract.[128,159] It also inhibits the absorption of glucose by suppressing the activity of disaccharidases[160] and maltase activity in intestine.[161] Additionally, the inhibitory effect of bitter melon on glucose absorption could be attributed to its high fiber content (48%) which facilitate slow absorption of glucose along the passage through gastrointestinal tract.[162,163]

The second pathway of anti-diabetic effects of bitter melon is the induction of insulin secretion. Our results clearly indicated a dose-dependent increase in insulin level in the diabetic rats treated with bitter melon. The results demonstrated an equivalent effect of bitter melon at dose of 400 mg/kg on insulin level with a classical insulin secretagogue; glibenclamide, while the higher dose (600 mg/kg) produce further elevation of fasting insulin level to be higher than control value. All of these results indicate the insulinotropic effect of bitter melon extract. Ahmed et al investigated the effect of daily oral administration of bitter melon fruit juice on the distribution of α, β and γ cells in the pancreas of STZ-induced diabetic rats using immunohistochemical methods which indicate an increased population of insulin secreting – β cells of endocrine pancreas.[164] Also, feeding rats with alcoholic extract of bitter melon showed definite improvement in the islets of Langerhans.[159] Physiological experiments have also shown that Bitter melon can stimulate insulin secretion from the endocrine pancreas and elicit glucose uptake in the liver.[165] The significant increase in serum insulin concentration of diabetic rats after bitter melon treatment in the present study might be ascribed to the ability of this agent to stimulate the spontaneous recovery of beta- cells of the islets of Langerhans. In vitro studies using isolated islets of Langerhans demonstrated that bitter melon induced a significant increase in insulin release. The work of Fernandes et al., (2007)[115], Garau et

al., (2003)[166] and Singh and Gupta (2007)[167] supports this finding. Treatment of diabetic rats with bitter melon showed a significant increase in β-cell number. This indicates that bitter melon has a regenerative effect on β-cells. Bitter melon may exert its effect by either preventing the death of beta cells by decreasing the oxidative stress in diabetic rats since bitter melon contains vitamin C (anti-oxidant) that neutralize the free radicals released.[159] Current evidence therefore indicates that the recovery and subsequent increase in the number of insulin producing cells followed by the release of insulin may be part of the several pathways by which bitter melon (M. charantia) exerts its hypoglycemic effects. In addition, bitter melon and its extracts may possess proliferative and growth properties similar to that of insulin.[168] Xiang et al.,(2007)[169] suggested that bitter melon may act as a growth factor for pancreatic beta cells.

Another important constituent of bitter melon which may play a role in inducing insulin secretion is the metal ion zinc. Zinc is needed by over 300 enzyme systems. Some of those are involved with the metabolism of blood sugar and are so important that a lack of zinc can cause type I or type II diabetes.[170] Zinc is highly concentrated in the insulin-secreting beta cells of pancreas and keep insulin molecules together in the beta cells. Beta cells must have zinc to function properly. We can suggest that the zinc content of bitter melon could play a role in the glucose-lowering effect through increasing insulin secretion. Also bitter melon contain high amounts of several amino acids that can stimulate insulin secretion. Most require glucose, but some, such as leucine, lysine and arginine, can stimulate insulin secretion in the absence of glucose,and therefore qualify as initiators of secretion. Leucine enters islets by a sodium - independent transport system and stimulates a biphasic increase in insulin release.[171] Gamma-amino butyric acid (GABA) is another important amino acids contained in bitter melon extracts which play an important role in modulating glucagon secretion. GABA is produced by pancreatic β cell and act on GABAA receptor in the α cells, causing membrane hyperpolarization and hence suppressing glucagon secretion. Also it was demonstrated that β cells also express GABA A receptors, forming an autocrine GABA signaling system. However, the role of this autocrine GABA signaling in the regulation of beta cell functions remains largely unknown.[172]

The present finding disagrees with the finding of Dans et al.,(2007)[173] who reported that bitter melon had no significant hypoglycemic and weight loss effects in alloxan diabetic rats. These contradictory data may be explained by different animal model of diabetes used. Dans et al used rats injected with alloxan which cause insulin-dependent diabetes through its ability to induce selective β-cell necrosis. This model is expected to not respond to insulin secretagogue agents and/or insulin sensitizers like bitter gourd while our model characterized by partial destruction of β-cell which retains ability to excrete insulin and also characterized by insulin resistance, all of which are a targets for correction by insulin secretagogue agents and/or insulin sensitizers.

Improving peripheral insulin resistance is another mechanism by which bitter melon corrects the diabetic state. Insulin resistance is a characteristic feature found associated with most cases of type 2 diabetes. In addition, insulin resistance is the hallmark feature of the metabolic syndrome. Insulin resistance results from the inability of insulin to produce its usual biologic actions because insulin interaction with its receptor fails to elicit downstream signaling events. The results of the present study indicated that, the diabetic rats suffer from insulin resistance (as indicated by increased HOMA) which is associated

with impaired insulin signaling in liver and muscles as indicated by lowered phospho-insulin receptor (Phospho-IR), IRS-1, PKC and Glut4.

The treatment of diabetic rats with bitter melon showed a dose-dependent increase of several components of insulin signaling in liver (P-IR and IRS-1) and muscle (P-IR, IRS-1, PKC and Glut4) with the doses from 100 to 400 mg/kg while the highest dose (600mg/kg) showed less efficient effect than the lower doses. This induction effect on the expression of these components of insulin signaling at the protein level may indicate that the constituents of bitter melon may act as a positive regulators at the transcriptional, post-transcriptional and/or translational level of gene expression, however the specific constituents responsible for these induction effect need further studies. With the exception of P-IR (active fraction of insulin receptor) the assay used in this study didn't differentiate between the active and inactive form of the signaling components studied. However, we can suggested that the increased insulin secretion activates autophosphorylation of insulin receptors at tyrosine residues (P-IR) which activates downstream IRS-1 pathways to activate PI3 kinase, PKB/Akt and PKC leading to translocation of Glut4 in the muscle from intracellular compartment into the membrane that enhance glucose uptake and utilization. In the liver the same pathway inhibits the activity of glycogen synthase kinase-3 β (GSK-3β) leading to enhanced glycogen synthesis and inhibited gluconeogensis. In line with our data, the protein analysis of insulin signaling pathway demonstrated that bitter melon supplementation to high fat diet (HFD)feed rats significantly increase the protein abundance of IR β, IRS-1, IRS-2, PI3 kinase and Glut4 in comparison with the untreated HFD group. Also, bitter melon supplementation significantly increased basal Akt phosphorylation as well as insulin-stimulated phosphorylation of IRS-1, Akt and PI3 kinase when compared with HFD animals.[174] Also an increasing Glut4 mRNA and protein expression level was documented in skeletal muscle of HFD rats treated with bitter melon.[175,176] Kumar et al (2009) reported a significant up-regulatory effects of the bitter melon extract on glucose uptake that associated with the induction of mRNA expression of Glut-4, PI3 kinase and peroxisome proliferator activator receptor gamma ($PPAR_{\gamma}$) in L6 myotubes.[116] Bitter melon extract exerted its effects similar to conventional insulin sensitizers, which act as a $PPAR_{\gamma}$ agonist that regulates genes involved in carbohydrate metabolism.[175] The Glut4 translocation and glucose uptake is mainly regulated by two pathways, one of them is the above mentioned insulin signaling pathway and the second one is the AMP-activated protein kinase (AMPK) pathway.[177]The triterpenoids of bitter melon was documented to activate AMPK with subsequent activation of glucose uptake.[117] From our study and other studies we can suggest that bitter melon fruit extracts act as insulin sensitizer and activate the glucose transport in a IR-IRS1-PI3 kinase-PKC-Glut4 dependent pathway.

The present study indicated that glibenclamide and bitter melon at dose of 400mg/kg produce comparable effect on insulin secretion; however, the peripheral response of insulin signaling components is better with bitter melon than glibenclamide (especially IRS-1 and Glut4). This observation may imply that bitter melon may contain constituents that may activate insulin signaling pathway apart from endogenous insulin. This can be explained by the presence of polypeptide-P or plant insulin (p-insulin) which is an insulin-like hypoglycemic protein, shown to lower blood glucose levels in gerbils and humans when injected subcutaneously.[178] The p-insulin works by mimicking the action of human insulin in the body and thus may be used as plant-based insulin replacement in patients with type-1 diabetes.[179]

There are several other pathway through which bitter melon extract induce anti-diabetic effects. It was documented that bitter melon extract contains a selective 11β -Hydroxysteroid dehydrogenase type 1 (11β -HSD1) inhibitor.[180] The finding provides an additional molecular mechanism for the widely recognized anti-diabetic effects of bitter melon, which most likely is the result of a concerted effect involving several active ingredients. 11β-HSD1is a microsomal enzyme that converts inert cortisone (dehydrocorticosterone in rodents) to active cortisol (corticosterone in rodents) and thus acts as an intracellular switch to mediate glucocorticoid action in metabolic tissues.[181,182] During the last decade, multiple evidences have accumulated that argues for an etiological role of 11β -HSD1 in obesity and type 2 diabetes.[183,184]

As expected in our model of diabetes, the disrupted glucose homeostasis is associated with derangements in the lipid profile manifested as increased triglycerides, total cholesterol and LDL-C, as well as decreased HDL-C (collectively called dyslipidemia). These derangements may be a cause or a consequence of the diabetic state and insulin resistance.[185] The treatment with bitter melon show triglycerides lowering effect at low doses (100 and 200 mg/kg) while the higher doses have no significant effect. Total cholesterol showed very mild correction with bitter melon treatment.While HDL-cholesterol showed dose-dependent increase, the LDL-cholesterol showed a dose-dependent decline with bitter melon extract at doses from 100 to 400 mg/kg while highest dose showed lesser effect. Also, glibenclamide showed partial correction of lipid profile.

Nerurkar et al., (2008) indicates that bitter melon juice lowers plasma apoB-100 and apoB-48 in HFD-fed mice by improving the phosphorylation status of insulin receptor and its downstream signaling molecule which ameliorate diabetic dyslipidemia.[186] Lipid-lowering properties of the bitter melon is documented in diabetic patients.[155] Bitter melon may affect the breakdown of specific lipoprotein (e.g LDL) or it may enhance fat oxidation in the body. The saponins and plant sterol in bitter melon also reduce blood triglyceride level and they also reduce the absorption of cholesterol from the intestine. In addition, the insulin like molecule in bitter melon may, like insulin, prevent the increase in triglyceride level due to the movement of fat from body cells into the blood stream.[187] Animal studies demonstrate bitter melon extracts, particularly the saponin fraction, have lipid-lowering effects resulting from inhibition of pancreatic lipase activity and subsequent decreased lipid absorption.[188] Several animal studies using a rodent model of diabetes have examined the effect of bitter melon extracts on abnormal lipid parameters. Significant decreases in triglycerides and LDL-cholesterol and increases in HDL-cholesterol were noted in the all studies.[189,190] After 10 weeks, diabetic rats receiving bitter melon extract experienced a normalization of all lipid parameters compared to control rats not given the extract.[191] Bitter melon treatment to old obese rats elicited a dose related hypolipidemic activity. All the lipid components *viz* cholesterol, LDL-cholesterol and triglycerides were reduced significantly. The more prominent effect being reduction in LDL- cholesterol which is a known triggering factor for coronary diseases. Considering bitter melon effect on the lipid components, it can be assumed a potential hypolipidemic agent, which will be of great advantage for diabetic condition as well as the associated atherosclerosis or hyperlipidemic conditions. [115]

The observed discrepancy between the effect of the highest dose of bitter melon (600mg/kg) and lower doses (100-400 mg/kg) need further investigation. However, we can suggest that bitter melon extract is a very complex mixture and may contains some minor

constituents which may have inhibitory effect on the insulin signaling and have no effect at low doses while at high dose these compounds could produce a significant effect. The results of the present study provide a support of this hypothesis because the highest dose of bitter melon produced the expected insulintrophic effect and the highest level of insulin was observed with this dose however; the fasting blood glucose showed greatly higher level than the lowest dose used and HOMA-insulin resistance index showed elevated values even higher than untreated rats. This insulin resistance state in the rats treated with the highest bitter melon dose was associated with low peripheral tissues contents of different components of insulin signaling which show values near that observed in the untreated diabetic rats. Another suggestion that could explain the low efficiency of high dose may be related to the environmental and/or agricultural contaminants for which the plant was exposed during breeding and processing. Such suggestions need further confirmation and presence of inhibitors or contaminants need to be confirmed. However, bitter melon is considered safe as an oral hypoglycemic agent, but blood glucose monitoring should follow. Bitter melon should be avoided in pregnant women, as it may cause a miscarriage, based on historical use and animal data. Bitter melon contain momorcharin and have been shown to have antifertility effects at high doses in female mice and spermatogenesis was inhibited in dogs after being fed bitter melon fruit extract for two months.[192]

From the above mentioned discussion and other related studies it is become clear that bitter melon extract is a powerful glucose and lipid -lowering factor that play important role as anti-diabetic treatment. Bitter melon produces its effect through multiple pathways includes, acting as insulin secretagogue, mimicking insulin action, induce peripheral tissues expression of the insulin signaling components and act as insulin sensitizer. The bitter melon extract at low doses produce anti-diabetic effects equivalent to classical sulfonylurea (glibenclamide) however, high dose is less efficient and may worsen the diabetic situation. So, bitter melon is recommended as food supplement or pharmacological extract to treat type 2 diabetes mellitus.