Mini Review: Bioassay of Anti-diabetic Plants on Serum Glucose Levels of Rats

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ABSTRACT

Since ancient times, plants have been a formal source of medicine; literature mentioned the use of plants in treatments of various human ailments. In recent world researchers are witnessing a vastly growing and renewed interest in complementary and alternative medicines. In particular, the herbal medicine market has exploded evolving from herbal shops and health food stores as well as pharmacies. With this increasing interest in phytomedicines, more individuals will explore the possibility of using herbal medicines to complement conventional antidiabetic therapy. The importance of antidiabetic plants in the development of economic and effective treatments for diabetes currently recognized by the World Health Organization which recorded that about more than 170 million people had diabetic diseases. Antidiabetic plants have often been used by practitioners of herbal medicine in treating individuals with non-insulin-dependent (type2) diabetes. In several traditional system of medicine, it is described that plants useful in diabetes also possess strong antioxidant / free- radical scavenging properties. Here, we aimed to review some data containing information for plants sources with hypoglycemic effect during recently periods, with related information including relevant references.

Keywords: Anti-diabetic plants, hyperglycemic and hypoglycemic effects, antioxidant activity, alloxan diabetic rats.

1. INTRODUCTION:

Since ancient times, plant remedies have been used to help to relieve diabetes. Subsequently many plants have been used through the world for treatment of diabetes. In view of this wide usage the expert committee on diabetes mellitus was investigated by Bnouham, *et.al* (2006). They reported a review of ten years of herbal medicine research (1990 ~ 2000). Investigate the effect of agents of plant origin used in traditional medicine that has a long folk history for the treatment of diabetes mellitus (Shoeib 1992, Saija, *et.al*, 1995; Gomes, *et.al*, 1995; Sharma *et.al*, 1997, Ahmed, *et.al*, 2000, Asano, *et.al*, 2001; Lee, *et.al*, 2002; and Tag El Din, *et.al*, 2006)

Several such plants show hypoglycemic activity when are taken orally, for example : *Allium cepa L, (Augusti, et.al, 1995); Morodica foetida L. (Marquis, et.al, 1977); Coccina indica L. (Khan, et.al, 1995); Cumium nigrum L. (Akhtar and Ali, 1991); Ficus bengalensis L. (Ali, et.al, 1998); Alium sativum L. (Ziyyat, et.al, 1997); Citrullus colocynthis L. (Al- Rowais, 2002); and Morus alba L. (Tag El Din, et.al, 2006).* However, Waddod et.al, (2007) showed some active compounds extracted from *Ficus recemosa L.* and *Acacia catechu L.* that are represented ones recipe causes an increase of serum insulin levels in alloxan diabetic rats possibly due to organization of pancreatic beta cells. While, Sama – Venkatesh, (2002) indicated that both aqueous ethanol and butanol extracts of *Helicters isora L.* roots has reduced glucose levels in blood of tested rats to 51 and 69%, respectively in prolonged treatment study. This antidiabetic effect may be acting by potentiating the pancreatic secretion or increasing of the glucose uptake. Draz *et.al* (2010) studied the hypoglycemic effects of chicory (*Cichorium intybus L*) herb in plasma rats, with feeding on standard diet supplemented with 10% chicory herb for diabetic rats. Their results showed significant decrease for plasma glucose and total cholesterol in diseased rats compared to diabetic control.

The aim of this review is to collate most available data on plant sources with hypoglycemic effect during recently periods, particularly the last twenty years by carrying out of bioassay of plants extract or its main components on diabetic rats in order to evaluate its hypoglycemic action.

2. WHAT IS DIABETES?

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, or action, or both. Diabetes mellitus, commonly referred to as diabetes was first identified as a disease associated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels of blood glucose hyperglycemia lead to spillage of glucose into the urine, hence the term sweet urine.

Normally, blood glucose levels are tightly regulated and entered the cells easily by insulin, a hormone produced by specialized cell (Beta cells) of the pancreas. After eating food, the blood glucose elevates and then the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down. Glucose is a simple sugar found in food. It is an essential nutrient that provides energy for the proper functioning of the body cells. Carbohydrates are broken down in the small intestine and the glucose in digested food is then absorbed by the intestinal cells into the bloodstream, and is carried by the bloodstream to all the cells in the body where it is utilized. However, glucose cannot enter the cells alone and needs insulin to aid in its transport into the cells. Without insulin, the cells become starved of glucose energy despite the presence of abundant glucose in the bloodstream. In certain types of diabetes, the cells inability to utilize glucose gives rise to the ironic situation of starvation in the midst of plenty. The abundant, unutilized glucose is wastefully excreted in the urine. It is important to note that even in the fasting state there is a low steady release of insulin than fluctuates a bit and helps to maintain a steady blood sugar level during fasting. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts a lifetime. (Bwititi & Musabayane, 1997 and Bwiliti, et.al, 2000).

3. WHAT CAUSES DIABETES?

Insufficient production of insulin (either absolutely or relative to the body's needs), production of defective insulin (which is uncommon), or the inability of cells to use insulin properly and efficiently leads to hyperglycemia and diabetes. This latter condition affects mostly the cells of muscle and fat tissues, and results in a condition known as "insulin resistance." This is the primary problem in type 2 diabetes. The absolute lack of insulin, usually secondary to a destructive process affecting the insulin producing beta cells in the pancreas, is the main disorder in type 1 diabetes. In type 2 diabetes, there also is a steady decline of beta cells that adds 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 as vigorously, hyperglycemia develops.

Diabetes occurs world-wide and the incidences of both type 1 and type 2 diabetes are rising; it is estimated that, in the year 2000, 171 million people had diabetes, and this is expected to double by 2030. This global pandemic principally involves type 2 diabetes, to which several factors contribute, including greater longevity, obesity, unsatisfactory diet, sedentary lifestyle and increasing urbanisation. Many cases of type 2 diabetes remain undetected. However, the prevalence of both types of diabetes varies considerably around the world, and is related to differences in genetic and environmental factors. (Ernst, 1997).

Generally, the nutrition system play an important role in diabetes mellitus, therefore schematic programs of nutrition meals showed be directed under supervision medicinal care. However, the protection and / or treatment against diabetes is usually performed using two ways:

1) Oral tablets are used to induce insulin secretion from β cells of pancrease in the beginning cases.

2) Injection insulin is used in the latter cases of diabetes mellitus (Bever and Zahnd, 1979 and Balbaa, 1981).

The major cases (about 80%) of diabetes diseases was attributed to normal insulin secretion but body cells can not used it efficiency and this high blood glucose levels was recorded. Dio, et.al (2001) proved that presence of positive relationship between the doses of antidiabetic substance and the plasma insulin secretion.

4. WHAT IS THE IMPACT OF DIABETES?

Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as microvascular disease. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases. This is referred to as macrovascular disease. Diabetes affects approximately 17 million people (about 8% of the population) in the United States. In addition, an estimated additional 12 million people in the United States have diabetes and don't even know it.

From an economic perspective, the total annual cost of diabetes in 1997 was estimated to be 98 billion dollars in the United States. During the same year, 13.9 million days of hospital stay were attributed to diabetes, while 30.3 million physician office visits were diabetes related. Remember, these numbers reflect only the population in the United States. Globally, the statistics are staggering. Diabetes is the third leading cause of death in the United States after heart disease and cancer.

5. EXPERIMENTAL BIOASSAY:

The experiments of many investigations have been carried out by Shalaby (1999), Pari and Umamaheswari (2000), Weber, et al (2002), Meselhy (2003), Zakir, et.al, (2004), Tag El Din, et al. (2006), Habiboddin, et al. (2007) Daraz, et al (2010).These techniques can be summarized as follows :-

1- The different tasted antidiabetic plants were ground individually then, soaked in petroleum ether to remove lipids. The defatted matter was extracted by ethanol 95%, followed by evaporation under vacuum to dryness of each extract.

2- Biological assay was conducted using male albino rats with weight ranged from 180 ~ 200 g. All rat groups were individually fed standard diets according to Campell (1961) or Al Nagdy (1970), as well as fed 10% glucose solution during the first 24h. The experiments were carried out to asses of the tested extract (20 mg / 100 g body weight, daily). However, Two main experiments were performed as the following:

   A) Normal non-diabetic rats were divided into several groups according to the numbers of the tested extracts, as well as normal control group. Blood samples were individually collected from each group in heparinised tubes at zero time (after 3h. which can be represented as control for this group), 7, 14, 21, 28 and 35 days after treatment. Blood samples were centrifugation at 3000 r.p.m for 15 min to obtain serum.

   B) Induced- diabetic rats can be obtained by intravenous injection of alloxan monohydrate (Sigma) in a single dose of 15 ~ 20 mg/100 g body weight dissolved in saline solution (Chen, et. Al., 2005) or streptozotocin (STZ, Sigma) in a single dose of 15 mg / 100 g body weight (Yang. et al., 2006). One group of diabetic rats was saved as diabetic control. The other groups were given individually the different tested extracts.

However, some of these investigations have been reported by Tag El Din, et.al,(2006) that used tested extracts of prickly-pear stems, leaves and stems, root barks of mulberry as food additive for 30 days. Whereas, Draz, et.al, (2010) used tested extract of chicory herb 10% as additive diet for 50 days.

On the other hand, advanced investigations for the antioxidant and antidiabetic properties were reported by Weber, et.al (2002) and Kar, et.al (2003). They fractionated the ethanolic extract into several ones on chromatographic column and used each fraction to fed the tested rats to assay the activity of each one as antioxidant and/or antidiabetic. However, the results revealed that the presence of positive relationship between antioxidant and antiabetic activities.

6. ANTIDIABETIC ACTIVITY:

Many investigation have been reported during the last twenty years concerning plants used as antidiabetic agent [Neef, et.al, 1995; Alarcon, et.al 1998 and 2000, Shalaby, 1999; Gray and Flet; 1999; Lemns, et.al, 1999, Lee et.al, 2001; Sama Venkatesh, 2002; Meselhy, 2003; Raba, et.al, 2004; Tag El Dind, et.al, 2006; Habiduddin, et.al, 2007; Ghosh, et.al 2008; Gutierrez, et.al, 2009 and Draz, et.al, 2010].

However, these studies evaluated the antidiabetic properties of several plants such as: Opuntia megantha L.; Morus alba L. Allium sativum L., Salvia officinalis L; Larrea Lagestroemia L.; Origanum compactum L; Camellia sinensis L.; Artemisia herbaalba L.; Chamaemelum nobile L.; Silybum marianum L.; Persea Americana L.; Sambucus nigra L.; Tilia Spp. L.; Turenera diffusal L. Taraxacum officinale L.; Trigonella fuenumgraecum L.; Psidium guajava L.; Agrimony eupatoria L.; Juniperus communis L.; Medicago sativa L.; Visca album L. Helicteres isora L.; Glossostemon bruguiri L.; Scoparia dulcis L.; Sclerocarya birrea L.; Rosmerinus officinalis L. Syzygium cuminil L. and Cichorium intybus L.

However, most sources are represented aromatic and medicinal plants. The functional antidiabetic parts may be included leaves or stems or roots and / or seeds (Barik, et.al, 2003; Aslan, et.al, 2004; Eidi, et.al, 2006, and 2007, Tag El Din, et.al, 2006; Guttierrez, et.al, 2009 and Draz, et.al, 2010). Other investigations showed that some plant extracts were used to exert hypoglycemic effect on diabetes such as Opuntia fuliginos L. (Trejo, et.al, 1996); Morus alba L. leaves (Bwitity and Musabayano, 1997); Prickly pear stems (Tag El Din, et.al, 2006); aqueous extract of Scoparia dulics L. (Dio, et.al, 2001); grab seeds Vitis virifera L. (Zakir, et.al, 2004) and mughat root wood Glossostemon burguiri L. (Meselhy, 2003). However, the hypoglycemic effect of these extracts are attributed a phytochemical compounds that play an important role to induce the insulin secretion by directly or indirectly ways as the following:

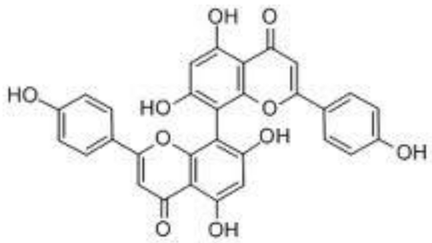
I. The medicinal photochemical compounds that extracted from most plant sources are mentioned previously such as gluconin, volatile oils, hormones, organo sulfer compounds, ricin, morobin, alkaloids, tannins, phenolic substances, sisqueterpins, tracant, terpinol, geraniol, linalool and saponin may be induced the insulin secretion, therefore blood glucose levels are decreased for the experimental rats. However, the medicinal compounds that extracted from these plant sources were discussed by Fernandez, et.al (1992 and 1994); Bwititi and Musabyane (1997); Shalaby, (1999); Kim, et.al (1999), Pari and Umamaheswari (2000), Dio, et.al (2001); Meselhy (2003) and Tag El Din, et.al (2006). They illustrated that most of physiochemical compounds that extracted from the previous sources induced insulin secretion. This directly hypoglycemic action was noticed by mught wood roots extract which caused decreasing about 55% and about 13% in blood glucose levels in the tested alloxan diabetic rats and non-diabetic rats when comparing to control, respectively (Shalaby, 1999). In addition, instant green tea powder was also used as an antidiabetic agent because it contained more than phenolics level which act as antioxidant and antidiabetic agents (Lee, et.al, 2002). Antidiabetic and in vivo antioxidant activity of ethanolic extract of Bacopa monnieri L. in aerial parts and a possible mechanism of action was reported by Ghosh, et.al (2008). The extract produced significant decrease in the blood glucose level when compared with the control in alloxan induced hyperglycemic rats and is comparable with standard drug glibenclamide. The extract prevented significant elevation of glycosylated hemoglobin in vitro. Thus, the extract might have insulin-like activity and the antihyperglycemic effect; it might be due to an increase in peripheral glucose consumption, as well as protection against oxidative damage in alloxanised diabetes. Other studies by Tag El Din, et.al (2006) illustrated the effect of prickly-pear stems and mulberry roots extract on serum glucose, urea and creatinine levels in alloxan – diabetic rats and non-diabetic ones. They showed presence of significant decrease in serum glucose level with an increase in body weight for non-diabetic rats after 20 ~ 30 days of treatment. They revealed also that flavonoids induced insulin secretion for both normal and alloxan – diabetic rats. The same effect was also noticed by using polyhydroxylated alkaloids (Asano, et.al, 2001). Other directly induction of insulin secretion was also discussed by Meselhy (2003) in mughat roots extract. He extracted a new biflavone moghatin (3 – hydroxycupressuflavone) together with five known compounds, which act as potant antidiabetic agent.

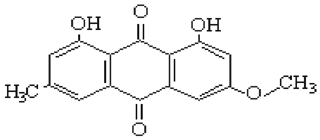
II. Stimulation of blood glucose levels in the tested animals by using various plant extract and / or some functional natural compounds by indirect pathway has been reported by Singh (1995) who indicated that ethanolic extract of B. monnieri L. contained rich amounts of saponin which act as antidiabetic agent (Yoshikawa, et.al, 1999) and also has antioxidant properties (Lee, et.al, 2005). However, saponins in ethanolic extract was confirmed to posses the activity that may be attributed to its protective action on lipid peroxidation, as well as the inhancing effect on cellular antioxidant defense contributing to the protection against oxidative damage in alloxanised diabetes (Haddad, et.al, 2003). In addition, aqueous ethanolic extracts of H. isora L. decreased the blood urea, triglygcerides, total cholesterol and glucose levels of the tested rats. The last one was attributed of increasing glucose uptake and thus glucose levels are decreased in the tested animals (kar, et.al, 2003). However, the positive relationship between antioxidant and antidiabetic activities was attributed to these tested extracts may be induce beta cells efficiency (Weber, et.al, 2002 and Singh, et.al, 2002). In addition, Weber, et.al (2002) showed that an aqueous methanolic extract of Psacalium peltatum L. roots exhibit hypoglycemic activity in the studied rats. Chromatographic separation showed that the 7th fraction significantly reduce blood glucose in the tested rats.

However, the potant antidiabetic effect was recorded for G. burguieri L. roots extract which decreased blood glucose level about 13.0 and 54.5% for the tested normal and diabetic rats, respectively when compared to control (Shalaby, 1999), while M.alba L. roots extract caused decrease of glucose levels about 16.0 and 61.5% for normal and diabetic rats, respectively at the end of 30 days after treatment (Tag El Din, et.al, 2006). Therefore it can be concluded that both of mulberry and mughat roots are represented a potant antidiabetic agents. While the most active antidabetic one was the aqueous butanol of H.isora L.roots (69%) as reported by Sama, Venkatesh (2002).

Chemical structure of some antidiabetic compounds:

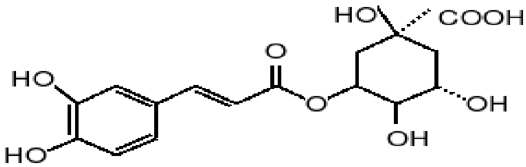
The roots of mughat (Glossosteman bruguieri L.) were rich of natural compounds had potant antidiabetic action (Meselhy, 2003). He isolated a new biflavone moghatin (3\\ – hydroxylcupressu – flavone) from the dried peeled roots of mughat, together with five known compounds such as 4 – methoxyisoscutellargin, sesamin, chrysophanal, emodine and methoxyemodin. The structures of these compounds were assigned on the basis of spectroscopic data after fractionation and repeated chromatographic separation for methanolic extract of mughat roots. The most antidiabetic action was the first compound (3\\\ hydroxyl cupressflavone), followed by methoxy emodine as the following structure:





3 hydroxy cupressuflavone Methoxy emodin

In addition, HPLC analysis of the M. alba L. ethanolic extract indicated the presence of many phytochemical compounds that are responsibled for antidiabetic, antihyperlipidemic and antioxidative effects. Eight of phenylpropane diglycerides, 3 flavones and 6 phenylpropanoid glucosides were detected. The most antidiabetic effect was utilized for one of the phenylpropanoid glucosides (chlorogenic acid) as the following:



Chlorogenic acid

However, their proposed structures were elucidated based on HPLC retention time, UV and MS profiles.
Meanwhile, a new HPLC – DAD – MS method was established for the identification and characterization of phenylpropanoid in ethanolic extract of Morus alba L. by Dio, et.al (2001). They indicated also that these phytochemical compounds caused decrease of serum glucose and total cholesterol levels and increase insulin secretion on the tested alloxan – induced diabetic rats.
Generally phytochemical examinations of most crude extracts showed the presence of flavonoids, tannins, terpins phenolic glucosides and

alkaloids, while saponins were detected only in mulberry stem and root bark extract (Tag El Din, et.al, 2006). Similar data reported by Perez, et.al, (1998); Yoshikawa, et.al (1999), and Meselhy, (2003). They showed the biological effect of antidiabetic plants can be attributed to the presence of saponin and phenolic glucosides that are extracted from M. albal and M. multiflora L., respectively.

7. HISTOPATHOLOGICAL EXAMINATIONS:

Histopathological examinations were carried out on pancreas and kidney tissues of alloxan-diabetic rats and non-alloxan diabetic ones treated with mulberry root bark extract. Pancreas tissue of induced alloxan – diabetic rats showed that the islet of langerhans cells were necrosis and surrounded by mononuclear inflammatory cells with congested blood vessels. In addition, islets seem to be smaller and less in number compared with normal rats.

Generally, plant extract treatments improved the islet of langerhans cells, where most cells tend to be normal in size but some of them were necrosis. Such effect was more noticeable in case of rats treated with mulberry root bark. The kidney cortex of untreated alloxan – diabetic rats represented sever hemorrhages and granular degenerative changes of the cells lining the renal tubules, others were necrosis. However, the treatment with plant extracts and\ or its active components induced slightly the improvements in kidney cortex cells, which decreased congested blood vessels and hemorranges (Munari, et.al, 1990, Bancroft et.al. 1994, Singh and Aggarwal, 1995, Lemus et.al, 2001, Amer et.al, 2004, Zakir et.al, 2004, and Tag El Din et.al, 2006). However, Sama – Venkatesh (2002) showed that histopathological examination of pancreas, liver and kidney showed the recovery of damage tissues when section of treated groups compared with diabetec control when used ethanol or butanol extracts of H. isora L. roots in the tested alloxan diabetic rats.

In conclusion, these survey have been mentioned some plants which are most effective and the most commonly studied in relation to diabetes, and their products i.e., active, natural principles and crude extract that have been used in traditional system of medicine. However, it remains important to determine the safety and efficacy of more antidiabetic plants and to understand their mode (s) of action.

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التأثير الحيوى للنباتات المضادة لمرض السكر على مستوى جلوكوز سيرم دم الفئران

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منذ قديم الزمان كانت النباتات مصدر أساسى للطب حيث ذكرت المراجع استخدام النباتات لعلاج الكثير من أمراض الإنسان. وأهتمت الدراسات الحديثة على تجديد الأهتمام بالعلاج المتكامل والمرتبط بالطب الحديث وبخاصة طب الأعشاب حيث أنتعشت سوق الأعشاب بوضوح فى الفترة الأخيرة فى محلات العطارة ومراكز الأغذية الصحية بالإضافة إلى تداولها فى الصيدليات. وبزيادة الأهتمام بطب النباتات فقد زاد الأهتمام بإكتشاف إمكانية

مساهمة طب الأعشاب فى العلاج التقليدى لمرض السكر خاصة بعد أن أشارت منظمة الصحة العالمية أن أعداد مرضى السكر قد تجاوز المائة وسبعون مليون فرد فى العالم وقابلين للزيادة.

واستخدمت هذة النباتات بواسطة المتخصصين فى طب الأعشاب فى علاج مرضى السكر من النوع الثانى non-insulin-dependent (type2) وهناك العديد من طرق العلاج التى أفادت أن لتلك النباتات دور آخر مهم ومفيد فى حالات مرضى السكر حيث تعمل هذة النباتات كمصدر غنى بالمواد المضادة للأكسدة والتى تعمل على إزالة الشقوق الحرة الضارة من الجسم ولها علاقة طردية مع النشاط المضاد لمرضى السكر.

وقد اهتم هذا البحث المرجعى بتوفير المعلومات عن أهم النباتات التى تعمل على خفض مستوى سكر الدم لمرضى البول السكرى. وكذلك الأشارة إلى التركيب الكيميائى لبعض المواد الفعالة فيها. هذا بالإضافة لذكر العديد من المراجع العلمية التى يمكن الرجوع اليها لإلقاء المزيد من الضوء عليها.

الكلمات المفتاحية: إنتاج الغاز معملياً – المصاصة ومحاصيل المحليات.