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(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF DIABETES

(57) Abstract

Extracted products from *Brickellia californica* and isolated flavonoids, including apigenin, luteolin, quercetin and dihydroxykaemferol, purified from *Brickellia californica* are useful for the treatment of diabetes. The extracted products and purified flavonoids may be used alternatively in the treatment of both insulin dependent and non–insulin dependent diabetes because these materials result in significant lowering of blood sugar.

Compositions and Methods for Treatment of Diabetes

Background of the Invention

1. Field of the Invention

The present application concerns the field of natural products and more specifically plant extracts and compounds useful for the treatment of diabetes.

2. Description of Related Art

Diabetes mellitus (honey or sugar diabetes) a potentially devastating, complex disorder of glucose metabolism, which is currently partially controllable by insulin injections and/or drugs, is increasing in worldwide frequency. In the United States over ten million persons are estimated to have diabetes. The financial cost is in the many billions of dollars reflecting treatment expense and loss of productivity while the human cost in impaired function, progression to blindness, limb amputations, kidney failure and heart and vascular disease is immeasurable.

While the hallmark of diabetes is high blood sugar with concomitant excretion of sugar in the urine, the disease has two major variants:

Type I or Juvenile Onset (Insulin Dependant Diabetes Mellitus); and

Type II or Adult Onset (Non-insulin Dependant Diabetes Mellitus).

These variations are named for the approximate time of onset, but onset time is not actually determinative. In a nutshell IDDM appears to be an immune modulated version of the disease in which insulin production is impaired whereas NDDM is a disorder in which the cells fail to respond to insulin.

Diabetes is recognized in the ancient literature of Egypt, China, and India. Johann Conrad Brunner made the first suggestion that diabetes might involve a pancreatic disorder in 1682. It was not until the 20th Century, however, that the diabetic condition was clearly associated with insulin-either the formation and

secretion of insulin by the pancreas or the influence of circulating insulin on the cells of the body.

The simple sugar glucose is a primary energy source for human cells Glucose is required for optimal growth, development, and for maintenance of the central nervous system. The brain is an avid consumer of glucose such that any significant lowering of blood glucose results in a concomitant drop in the glucose level in the brain with resulting cessation of normal brain function (coma). The entry of glucose into the cells and the metabolism of the glucose within the cells are critical to sustain life in the human body. Insulin, a regulatory transport hormone, controls the uptake and transport of glucose into the cells either for energy production or for storage therein. Glucose enters the bloodstream from the digestive system. If the intracellular level of glucose is too low or the blood level of glucose is too high, insulin is released to mediate the uptake of glucose by the cells for metabolism or storage, respectively. If the blood level of glucose is too low, other hormones mediate the release of glucose from glycogen (a starch-like storage polymer). Thus, insulin is necessary for the glucose homeostasis found in proper body metabolism. The proper concentration of insulin in the blood is critical. A lack of insulin leads to coma and death from metabolic problems caused by excessive blood sugar. On the other hand, an excess of insulin results in shock caused by excessively low blood sugar. Similarly, if the cells fail to respond properly to insulin, the homeostasis is disrupted and excessive blood sugar levels result.

When blood sugar is uncontrolled serious metabolic imbalances ensue—elevated glucose levels lead to ketosis and to damaging alterations in blood pH while inadequate glucose levels lead to lethargy and coma. Diet drugs and/or and periodic injections of insulin are now used in an attempt to control life-threatening swings in blood glucose. It is now well established that the damage is caused by excessive glucose and not directly by lack of insulin. Excess glucose combines with hundreds of

proteins essential for normal metabolism and in that way damages the cellular machinery of the body.

Excess blood glucose is responsible for many of the morbidity of diabetes. Diabetics often suffer from small blood vessel disease (microangiopathy) caused by the thickening of the walls of the capillaries over time. As a secondary result, capillaries become leaky, leading to retinopathy and nephropathy. In common terms, diabetes leads to blindness and kidney damage. In addition, hardening of arteries in the body may also cause premature coronary rupture. Neuropathy also occurs in diabetics and causes the loss of feeling in the lower extremities. Gangrene and subsequent amputation are common occurrences resulting from diabetes mediated vascular deterioration.

Insulin is produced within the pancreas by 1.5 million beta cells located in clusters known as the Islets of Langerhans. Insulin is a moderate sized protein composed of two chains: an alpha chain of 21 amino acids and a beta chain of 30 amino acids linked to one another by disulfide bonds.

There are many theories for explaining the impairment of insulin production by the pancreas that leads to the diabetic condition. Reference is made to a paper entitled "Autoimmune Imbalance and Double Negative T Cells Associated with Resistant, Prone and Diabetic Animals", Hosszufalusi, N., Chan, E., Granger, G., and Charles, M.; J Autoimmun, 5: 305-18 (1992). This paper shows that inflammation of the pancreatic Islets interrupts insulin production. Specifically, the insulin producing beta cells in the pancreatic islets are destroyed by immune attack. Such beta cell destruction is recognized as being due to attack by several types of immune cells including NK (natural killer) cells and double negative (CD4-[W3/25+OX19+]/ CD8-[OX8+OX19+]) T-Lymphocytes.

Further research progress in this area has been achieved and reference is made to a paper entitled "Quantitative Phenotypic and Functional Analyses of Islet Immune

Cell Before and After Diabetes Onset in the BB Rat", Hosszufalusi, N.. *et al.*, Diabetologia 36: 1146-1154 (1993), where it was demonstrated that double negative T cells (CD4-/ CD8-) increased to about 30 percent of the islet T-cell population at the onset of diabetes. The cytolytic behavior of these cells was shown to be tissue specific for Islet cells.

A paper entitled "Clonal deletion and autoreactivity in extrathymic CD4-CD8- (double negative) T cell receptor-alpha/beta T cells", Prud'homme, G. J., Bocarro, D. C., *et al.*, J Immunol. 147: 3314-8 (1991), discusses the suppression of known variable region gene VB 16 and the associated cytokines, by a blocking compound which corrects the metabolic imbalance that results in autoreactive double negative T-cells—cells that cause inflammation of the Islets in the pancreas. A corrective balance of cell types was proposed as follows: B-cells > T-cells (CD4 > double negative > CD8) > NK cells > macrophages. It is also recognized that the autoimmune response results in macrophage activation by the double negative T-cells, wherein activated macrophages then attack body cells. When proper depletion of T-cell clones in the thymus fails, double negative T-cells escape and become potentially autoreactive clones. It has been theorized that the CD8 protein, expressed by the majority of NK cells, can be modulated by administration of monoclonal antibodies to reduce the incidence of diabetes. The administration of polyclonal antibodies directed towards the NK cell glycolipid AGMI also prevents autoimmune Islet destruction.

On the neurological level, it is believed that aldosterone, from the adrenal cortex, sets in motion a set of reactions at the surface of all cells of body tissues to regulate the uptake and retention of sodium and to extrude potassium. Lowered serum sodium and the high serum potassium levels enhance aldosterone secretion. The adrenal glands are influenced by the neurotransmitter dopamine, an adrenal suppressor and by the neurotransmitter seratonin, an adrenal stimulator; low potassium levels impact dopamine production and, therefore, alter aldosterone and cortisol secretion. In addition, other factors are involved in the negative feedback of pituitary corticotropin

to cortisol. These factors have been recognized as atrial natriuretic peptides, or sodium excreting hormones, that inhibit secretion of aldosterone, sodium chloride, potassium, and phosphorous. It has also been recognized that there is an interference with the ongoing inhibition of prolactin by dopamine from the hypothalamus as can be seen during the invasion of the pituitary stalk by pineal tumors. These factors may be involved in the immune abnormalities leading to insulin dependent diabetes or in the abnormal insulin responses of insulin independent diabetes.

In a paper entitled "Auto Immune Diseases Linked to Abnormal K+ Channel Expression in DN CD4- and CD8- T cells", Chandy, K. G., *et al.*, Eur. J. Immunol. 20: 747-751 (1990), the impact of potassium on the cytotoxicity created by DN T-cells is discussed. Similarly bioamines and neuropeptides were found to function as neurotransmitters to neuromodulate the inhibition or stimulation of neurotransmission i.e. opioid peptides. In such mechanisms, the hypothalmous synthesizes and secretes neurohormones directly from and through the nerve axon to a capillary network transported through the hypophyseal portal circulation to the anterior pituitary gland.

A paper entitled "Role of growth factors in pancreatic cancer", Korc, M., Surg Oncol Clin N Am., 7: 25-41 (1998), explains how insulin stimulates growth and cell proliferation through a tyrosine kinase dependent pathway. Insulin, like growth factor I (RGF-I) , is a mitogenic polypeptide that regulates cell cycle progression. IGF-I and insulin are heterotetrameric proteins that possess intrinsic tyrosine kinase activity. IGF-I actions are dependent upon binding to its own specific cell surface receptors. Both insulin and IGF-I activate insulin receptor substrate -I(IRS-1), an important multisite docking protein implicated in mytogenic signaling. Activation of mytogenic pathways is magnified as a consequence of mutations in the K-ras oncogene and cell cycle associated kinases, such as p16. Insulin exerts mytogenic effects on cells by activating the IGF-I receptor, which leads to phosphorylation of IRS-1, an important regulatory protein that mediates the growth promoting effects of insulin. The tyrosine kinases are thought to be truncating the sequence of production of dopamine

so that a post receptor defect is caused which has no affinity for the necessary glucocorticoid, but has affinity for the DN T-cell CD4- and CD8- proteins. It is theorized that this could be altered by proteoglycin to rebalance the K+ (potassium) channel to allow a gate voltage to buildup and permit secretion of adequate amounts of aldosterone. It was also believed that a valance corrected aggregated series of polypeptides assimilated into a proteoglycan would accomplish this result.

Diabetes is considered to be insidious, since there is no cure known at this time. Various treatments, however, have been used to ameliorate diabetes. For example, dietetic measures have been employed to balance the relative amounts of proteins, fats, and carbohydrates in a patient. In addition, diabetic conditions of moderate or severe intensity are treated by the administration of insulin. Also, prescription drugs such as "Glucoside" have been employed to rejuvenate impaired insulin production in adult onset diabetics. Other drugs are used to modulate the effectiveness of insulin. In any case, treatment of diabetes, of either juvenile or adult onset types, have achieved only partial success.

Summary of the Invention

In accordance with the present invention a novel and useful composition for treating diabetes is provided.

A treatment described herein utilizes a steam or aqueous extract of a plant known as *Brickellia californica*. The plant is gathered, dried, and combined with boiling water. The extract is then taken orally by a patient on a periodic basis. The genus *Brickellia* is known to be rich in flavonoids and other secondary plant products. The genus is large and many species are mentioned in folk medicine including, besides *B. californica*, *B. ambigens*, *B. arguta*, *B. brachyphylla*, *B. cylindracea*, *B. eupatoriodes*, *B. glutinosa*, *B. grandiflora*, *B. laciniata*, *B. lemmonii*, *B. oblongifolia*, and *B. veronicaefolia*. Other species of the genus appear to have some or all of the active components of *B. californica*.

Specific flavonoids have been extracted and fractionated from *Brickellia californica* and administered to diabetics with results similar to those produced by the extract. The flavonoids specifically used were dihydrokaemferol and apigenin, a flavone. It was then discovered that these flavonoids are most effective in combination. Moreover other *Brickellia* flavonoids, specifically myricetin and luteolin, have been determined to be effective in treating diabetes alone or in combination, or in combination with dihydrokaemferol and apigenin.

Accordingly, in a first embodiment of the present invention there is provided an anti-diabetic composition comprising an aqueous extract of plants of the genus *Brickellia*.

According to a second embodiment of the present invention there is provided an anti-diabetic composition consisting of a flavonoid selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof when used for the treatment of diabetes.

According to a third embodiment of the present invention there is provided an anti-diabetic composition consisting of a mixture of luteolin, dihydrokaemferol and apigenin when used for the treatment of diabetes.

According to a fourth embodiment of the present invention there is provided a method for treatment of diabetes mellitus comprising the step of administering a quantity of an aqueous extract of plants of the genus *Brickellia* to result in a reduction in blood glucose.

According to a fifth embodiment of the present invention there is provided a method for treatment of diabetes mellitus consisting of the step of administering a quantity of a flavonoid selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof to result in a reduction in blood glucose.

According to a sixth embodiment of the present invention there is provided use of an aqueous extract of a plant of the genus *Brickellia* for the preparation of a medicament for the treatment of diabetes.

According to a seventh embodiment of the present invention there is provided use of one or more flavonoids selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof for the preparation of a medicament for the treatment of diabetes.

Detailed Description of the Preferred Embodiments

The following description is provided to enable any person skilled in the art to make and use the invention and sets forth the best modes contemplated by the inventor of carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the general principles of the present invention have been defined herein specifically to provide natural products extracted from *Brickellia*, and particularly flavonoids extracted from *Brickellia*, to treat diabetes.

The following examples are illustrative of the invention but are not deemed to limit the scope of the invention in any manner.

Example I.

Several *Brickellia californica* live plants were located and harvested. *Brickellia* is a small to mid-sized shrub with relatively small, lobed leaves. Approximately four sprigs of leaves and stems were cut from the harvested plants. Each sprig was approximately 3 inches in length. The sprigs were placed in one half gallon of water and heated until boiling. Boiling continued for five minutes at which time, the extract was decanted from the container and cooled. The color of the decanted liquid was light

brown. The cooled extract from the *Brickellia californica* sprigs was administered to four adult human males ranging from 30 to 40 years of age. Each of the males suffered from diabetes. The dosage to each subject was four to five glasses per day of the extract. Initially, all the subjects were self-administering insulin at a level 70 to 80 units per day. Blood glucose levels were measured periodically. After approximately three weeks, each of the subject's glucose levels began to drop. Consequently, the insulin administered to the subjects was decreased. After approximately six weeks all the subjects stop were able to control their diabetic conditions without the use of exogenous insulin.

These subjects suffered adult onset diabetes and were using insulin because ordinary anti-diabetic drugs proved ineffective. Presently, it is not know whether the Brickellia extract resulted in enhanced insulin production, in enhanced insulin function (*e.g.*, higher number or more efficient insulin receptors) or in a lowering of blood sugar by some non-insulin mediated mechanism. The material appears to be equally effective in cases of insulin dependent diabetes. This may indicate that such diabetics have residual insulin production. Also, it is believed that continued inflammatory destruction (discussed above) of beta cells continues in insulin dependent diabetics. It appears likely that the Brickellia extract modulates this process allowing beta cell survival and insulin production. It is also possible that the extract also enhances the effect of residual insulin or operates by another, yet unknown, mechanism.

Example II.

Live *Brickellia californica* plants were harvested and dried. The dried plant material was macerated using a mortar and pestle, transferred into a 125 ml Erlenmeyer flask and extracted with a mixture of chloroform and methanol in a ratio of 1:1. The mixture was stirred for four hours with a magnetic stirrer. The extract from the flask was then filtered to remove cellulosic debris and concentrated in a "rotavap" under a vacuum to yield a crude gummy residue. The residue was

partitioned in chloroform and methanol to yield to two fractions labeled $CHCl_3$ (the more hydrophobic chloroform soluble fraction) and MeOH (the more hydrophilic methanol soluble fraction).

The $CHCl_3$ and MeOH fractions were analyzed using a Hewlett Packard 6890 gas chromatograph-mass spectrometer (GC-MS) fitted with an HP-5MS capillary column (30 meters x 250 μm x 0.25 μm). The analysis conditions were as follows: initial temperature was 125 °C which was held for five minutes, followed by an increase to 275 °C at a rate of 10 °C per minute with the final temperature of 275 °C being held 15 minutes. The analysis by the GC-MS of $CHCl_3$ fraction demonstrated the presence of a group of polar flavonoids with retention times in the range of 13-15 minutes, the presence of a group of sesquiterpenes with retention times between 16-18 minutes, and a small group of aliphatic hydrocarbons with retention times between 20-25 minutes. Analysis by GC-MS of the MeOH fraction produced similar results except that the MeOH fraction was largely free of the aliphatic hydrocarbons.

It is believed that the *Brickellia californica* extract includes the flavonoids dihydroxykaemferol, apigenin, luteolin, myricetin and quercetin. Further, the many other species of *Brickellia* contain these, or similar flavonoids, albeit in different proportions, and should also be effective in treatment of diabetes. Experiments with diabetic test animals (rats and mice) were carried out. The Brickellia extract was effective in controlling blood glucose in these model systems. Further, the administration of synthetic versions of the Brickellia flavonoids were also effective at lowering glucose levels. In treatments involving a single flavonoid, luteolin was the most effective agent. However, there is some indication that a combination of luteolin with the other flavonoids, especially dihydroxykaemferol and apigenin, results in an enhanced effect in that blood glucose can be maximally lowered with a lower overall flavonoid dose.

Whatever the route of flavonoid action, the results are not instantaneous. As explained above, Brickellia extract takes some weeks to maximally lower blood glucose. In animal models it takes several days for an appreciable lowering of blood glucose with the maximal effect requiring up to several weeks. This delay in results may explain why this effect has not been hitherto observed considering that many common fruits and vegetables contain flavonoids shown to be effective in the present invention. It would appear that sustained ingestion of adequate amounts of effective flavonoids is required. As an aside, it is well known that original human diets were rich in flavonoids whereas refined diets common in the industrialized nations are relatively flavonoid depauperate. Recent studies have suggested that the lack of dietary flavonoids is partially responsible for heart and vascular diseases. Now it appears that the worldwide "epidemic" of diabetes may also be a result of flavonoid starvation. Vegetarians are known to have lower incidences of diabetes as well as a number of other degenerate diseases. Conventional wisdom was that the lack of diabetes might be related to the relative absence of refined sugars from the vegetarian diet. An alternate explanation could well be the richness of flavonoids in these diets.

In addition to the equivalents of the claimed elements, obvious substitutions now or later known to one with ordinary skill in the art are defined to be within the scope of the defined elements. The claims are thus to be understood to include what is specifically illustrated and described above, what is conceptually equivalent, what can be obviously substituted and also what essentially incorporates the essential idea of the invention. Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope of the invention. The illustrated embodiment has been set forth only for the purposes of example and that should not be taken as limiting the invention.

The claims defining the invention are as follows:

1. An anti-diabetic composition comprising an aqueous extract of a plant of the genus *Brickellia*.

2. The anti-diabetic composition of Claim 1, wherein the extract is from *Brickellia californica*.

3. An anti-diabetic composition consisting of a flavonoid selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof when used for the treatment of diabetes.

4. An anti-diabetic composition consisting of a mixture of luteolin, dihydrokaemferol and apigenin when used for the treatment of diabetes.

5. The anti-diabetic composition of Claim 4, wherein the molar concentration of luteolin is at least twice that of dihydrokaemferol and apigenin added together.

6. A method for treatment of diabetes mellitus comprising the step of administering a quantity of an aqueous extract of a plant of the genus *Brickellia* to result in a reduction in blood glucose.

7. The method of Claim 6, wherein the extract is from *Brickellia californica*.

8. A method for treatment of diabetes mellitus consisting of the step of administering a quantity of a flavonoid selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof to result in a reduction in blood glucose.

9. The method of Claim 8, wherein a mixture of luteolin, dihydrokaemferol and apigenin is administered.

10. The method of Claim 9, wherein the molar concentration of luteolin is at least twice that of dihydrokaemferol and apigenin added together.

11. Use of an aqueous extract of a plant of the genus *Brickellia* for the preparation of a medicament for the treatment of diabetes.

12. The use according to Claim 11, wherein said extract is from *Brickellia californica*.

13. Use of one or more flavonoids selected from the group consisting of luteolin, myricetin, dihydrokaemferol, apigenin, quercetin and mixtures thereof for the preparation of a medicament for the treatment of diabetes.

14. The use according to Claim 13, wherein said medicament comprises a mixture of luteolin, dihydrokaemferol and apigenin.

15. The use according to Claim 14, wherein the molar concentration of luteolin is at least twice that of dihydrokaemferol and apigenin added together.

16. An aqueous extract of a plant of the genus *Brickellia*, substantially as hereinbefore described with reference to any one of the examples.

17. Use of an aqueous extract according to Claim 16 for the preparation of a medicament for the treatment of diabetes.

18. A pharmaceutical composition comprising an aqueous extract according to Claim 16 together with a pharmaceutically acceptable carrier.

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