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Biguanide related compounds in traditional antidiabetic functional foods

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ABSTRACT

Biguanides such as metformin are widely used worldwide for the treatment of type-2 diabetes. The identification of guanidine and related compounds in French lilac plant (*Galega officinalis* L.) led to the development of biguanides. Despite of their plant origin, biguanides have not been reported in plants. The objective of this study was to quantify biguanide related compounds (BRCs) in experimentally or clinically substantiated antidiabetic functional plant foods and potatoes. The corrected results of the Voges–Proskauer (V–P) assay suggest that the highest amounts of BRCs are present in green curry leaves (*Murraya koenigii* (L.) Sprengel) followed by fenugreek seeds (*Trigonella foenum-graecum* L.), green bitter gourd (*Momordica charantia* Descourt.), and potato (*Solanum tuberosum* L.). Whereas, garlic (*Allium sativum* L.), and sweet potato (*Ipomea batatas* (L.) Lam.) contain negligible amounts of BRCs. In addition, the possible biosynthetic routes of biguanide in these plant foods are discussed.

1. Introduction

Type-2 diabetes is the most common metabolic disorder worldwide, and its prevalence is growing at alarming rates in both developed and developing countries. Oral antidiabetic agents commonly available on the market to treat type-2 diabetes include biguanides, sulphonylureas, thiazolidinediones, meglitinides and α-glucosidase inhibitors (Mahajan & Gupta, 2010). Among biguanides, metformin (1,1-dimethylbiguanide), also known as 'glucophage', is the most widely prescribed drug used in the treatment of diabetes. The history of metformin can be traced back to the use of French lilac (goat's rue, Spanish sanfoin, or false indigo) as herbal medicine in medieval Europe. French lilac is rich in guanidine, a substance with blood glucose-lowering (hypoglycaemic) activity that is present in the basic structure of metformin (Bailey & Day, 2004) (Fig. 1). Similarly, indigenous herbal medicines have been used in the treatment of diabetes in Ayurvedic medicine practiced in India by Charaka and Sushruta since 6th century BC (Grover, Yadav, & Vats, 2002). Unlike guanidine, biguanides (such as metformin) are non-toxic to animals and safe for clinical use in diabetic patients (Bailey & Day, 2004). Because biguanides do not increase pancreatic insulin secretion, they are referred to as antihyperglycaemic agents as opposed to hypoglycaemic agents (sulphonylureas). Biguanides reduce hyperglycaemia by increasing insulin sensitivity, decreasing glucose absorption, and inhibiting hepatic gluconeogenesis. Metformin is currently available in the drug market because of its unique mechanism of action, lower risk of lactic acidosis as compared to phenformin, and its successful use in over 90 countries. Suppression of hepatic glucose production and increased peripheral insulin sensitivity appear to be the major mechanisms of action by which metformin restores glycaemic control (Goo, Carson, & Bjelajac, 1996). In addition, biguanides are also used as antimalarial drugs. Furthermore, these compounds are known to have oral hypoglycaemic, tumor-inhibiting, antibacterial, tuberculostatic and antiviral properties (Kurzer & Pitchfork, 1968). The less toxic guanidine-containing compound, galegine is also an antidiabetic compound isolated from French lilac (Bailey & Day, 2004). Galegine is known for its weight-reducing properties indirectly by inhibiting synthesis and stimulating oxidation of fatty acids (Mooney et al., 2008). L-Arginine is a guanidine-containing amino acid known to stimulate the release of hormones, such as insulin, glucagone, prolactine and growth hormone (Tousoulis et al., 2002). Interestingly, the structure of guanidine is similar to that of urea (Barritt, 1936). Biuret which is a byproduct of urea is structurally similar to biguanide.

Plants have been the source of medicinal treatments for thousands of years. They play an essential role in the primary health care of 80% of the world's developing and developed countries (King, Aubert, & Herman, 1998). Many of the currently available drugs have been directly or indirectly derived from plants. Out of an estimated 250,000 higher plants, less than 1% have been exploited pharmacologically and even less in regard to diabetes. There are about 800 plants that may possess potential antidiabetic ingredients. Functional plant foods such as fenugreek, curry leaves, bitter gourd, garlic, and sweet potato are few among 45 plants that have shown experimental or clinical antidiabetic activity (Grover

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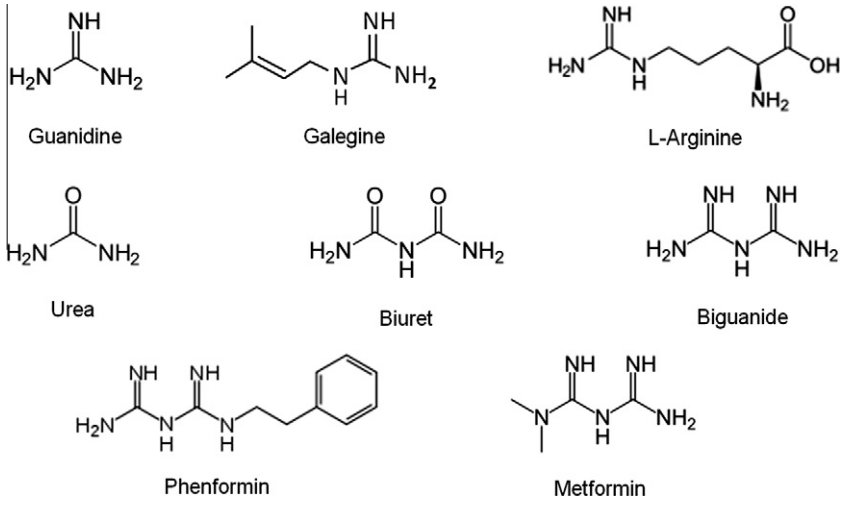

Fig. 1. Biguanides and related compounds. Guanidine is the basic unit of biguanide and has antidiabetic properties. It is structurally similar to urea. Biuret, a byproduct of urea, is structurally similar to biguanide. Galegine is an antidiabetic guanidine compound identified in French lilac known to have weight reducing effects. L-Arginine is a guanidine containing amino acid known to stimulate the release of insulin hormone. Metformin and phenformin are biguanides used as oral antihyperglycaemic drugs for type-2 diabetes treatment.

et al., 2002). Alkaloids, glycosides, galactomannan, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, flavonoids, phenolics, amino acids and inorganic ions are a few of the plant-derived compounds that have demonstrated antidiabetic activity (Grover et al., 2002; Jung et al., 2006). The following active ingredients were found to be effective in controlling glucose levels in various diabetic experimental models (rats, rabbits, dogs, gerbils, langurs or humans): allicin and its sulphur containing amino acid precursor from garlic; polypeptide-p, momordicin, charantin (insulin like protein called plant insulin), kakara-1b, -111a and -111b, galactose-binding lectin and oleanolic acid 3-*O*-glucuronide from bitter gourd; and alkaloid trigonelline, furostanol saponins (trigoneosides glycoside D and trigofaenoside A), steroidal sapogenins (diosgenin and yamogenin), and 4-hydroxyisoleucine from fenugreek (Grover et al., 2002; Saxena & Vikram, 2004). Some flavonoids, polyphenols, and their sugar derivatives have been reported to be effective against the inhibitory activities of α-glucosidase and aldose reductase (Jung et al., 2006).

Although, fenugreek, curry leaves, bitter gourd, garlic and sweet potato are known for antihyperglycaemic properties in Ayurvedic medicine for several centuries, detailed list of active components remain to be determined. The concept of synthesising biguanides, such as metformin, originated from the hypoglycaemic properties of plant guanidines. However, there has been no report on plant biguanides. The objective of this study was to quantify biguanide related compounds (BRCs) in functional plant foods (fenugreek, curry leaves, bitter gourd, garlic and sweet potato) and potato tubers. The presence of antidiabetic compounds, such as flavonoids and polyphenols, in potato tubers encouraged us to include them in this study (Perla, Holm, & Jayanty, 2012).

2. Materials and methods

2.1. Plant samples

Dry fenugreek seeds (*Trigonella foenum-graecum* L.; Fabaceae family; 200 g), fresh green curry leaves (*Murraya koenigii* (L.) Sprengel; Rutaceae family; 170 g) and fresh green bitter gourds (*Momordica charantia* Descourt.; Cucurbitaceae family; 25 fruits) were purchased from Asian Indian Grocery store, Denver, Colorado. Garlic cloves (*Allium sativum* L.; Amaryllidaceae family; 16 bulbs) and orange fleshed sweet potatoes (*Ipomoea batatas* (L.) Lam.; Convolvulaceae family; 6 tubers) were purchased from local markets. White-fleshed potatoes (*Solanum tuberosum* L. cv. Rio Grande Russet; Solanaceae family; 5 tubers) were collected from stored tubers harvested in September, 2010 at San Luis Valley Research Center. All the food samples, with the exception of fenugreek seeds, were freeze-dried at 0.12 mBar vacuum and −50 °C in a laboratory freeze-dryer (FreeZone 6, Labconco, Kansas City, MO). Freeze-dried samples and fenugreek dry seeds were ground to a fine powder in a coffee-grinder and stored separately in air-tight plastic zipper bags at −80 °C until further analysis.

2.2. Extraction of BRCs

Each plant food was extracted three independent times using a method that was previously adopted for animal plasma with modifications (Freedman, Blitz, Gunsberg, & Zak, 1961). In each extraction, 15 ml of 20% NaCl was mixed with 1.67 g of powdered sample in a 250 ml capacity glass bottle and shaken horizontally at 250 rpm for 30 min at 25 °C. To this mixture, 3.75 ml of 50% trichloroacetic acid was added and mixed by vortex. After 10 min at room temperature, 190 ml of chloroform–methanol (85:15, v/v) and 7.6 ml of 10 N NaOH were added and the mixture was shaken horizontally at 150 rpm for 25 min at 25 °C. The mixture was then centrifuged at 1960g for 10 min at room temperature. After aspirating and discarding the top aqueous phase and debris, the chloroform extract was collected in 1 l glass bottle. Three independent chloroform extractions from each food sample were pooled together in a 1 l bottle, reduced by evaporation to approximately 30 ml under a stream of nitrogen gas in a hood, and filtered with Whatman 40 filter paper. The concentrated chloroform extract was acidified with 0.4 ml of an acidic ethanol solution (2 N HCl in 95% ethanol) and evaporated to dryness under a stream of nitrogen gas in the hood. The dried extract was dissolved in 6 ml of a 5% NaCl solution by vortex and diluted with 6 ml of distilled water. After filtering, the final volume of the extract was adjusted to 12.5 ml with 2.5% NaCl solution and stored at −20 °C until further use.

A zeolite base-exchange column was also used to isolate BRCs from fenugreek samples according to the procedure reported by Freedman et al. (1961) with modifications. Zeolite was purified according to the procedure of Freedman et al. (1961) and dried