ORIGINAL ARTICLE

IN VITRO HYPOGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT OF SOME INDIGENOUS PLANTS

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ABSTRACT

Pakistan is rich in medicinally important plants and has ancient herbal treatment methods. Present work is based on the study of six indigenous plants *Eugenia jambolana, Lawsonia inermis, Momordica charantia, Morus*

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alba, Nigella sativa and *Trigonella foenum graecum* which show the inhibitory effect of glucose utilization, and are in use as hypoglycemic agents of varying degree in traditional system of medicine. The glucose uptake activity of (methanolic extracts) of these plants was tested *in vitro* and glucose was estimated by glucose oxidase method. The results in three different media revealed that, hypoglycemic activity is more prominent in neutral and basic media as compared to acidic medium.

Keywords: *Eugenia jambolana, Lawsonia inermis, Momordica charantia, Morus alba, Nigella sativa* and *Trigonella foenum graecum.*

INTRODUCTION

Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) (WHO/Acadia, 1992). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of the body systems, particularly the blood vessels and nerves (Nagappa *et al.,* 2003). For its therapy, along with the synthetic drugs, many agents of the plant origin are also in use particularly for the treatment of non-insulin dependent diabetes mellitus (NIDDM).

Plants are always an exemplary source of drugs, in fact many of the currently available drugs were derived either directly or indirectly from them. According to world ethnobotanical information reports, almost 800 plants may possess antidiabetic potential (Alarcon-Aguilara *et al.,* 1998). In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin and screening of more effective and safe hypoglycemic agents has continued to be an important area. In developing countries 80% of population are using traditional medicine in primary medical problems (Grover and Yadav, 2004). However, lots of herbs are now being used in the management of DM. Pakistan is endowed with the wealth of medicinally important plants and has ancient herbal treatment methods where traditional alternative medicines are popularly practiced among the large segment of its population.

Our work is based on the study of some indigenous plants which show inhibitory effect of glucose utilization, and are in use as hypoglycemic agent in traditional system of medicine. *Momordica charantia* (MC), *Eugenia jambolana* (EJ), *Lawsonia inermis* (LI), *Morus alba* (MA), *Nigella sativa* (NS) and *Trigonella foenum graecum* (TF) have been shown to possess hypoglycemic activity of varying degree. The results in three different media revealed that, hypoglycemic activity is more prominent in neutral and basic media as compared to acidic medium.

Momordica charantia

Momordica charantia (MC) is one of the plant that has been frequently used as medicine (Giron, *et al.*, 1991; Lans and Brown 1998; Joshi 2000; Sastri and Venkataraman, 1962). In India, various medicinal properties are claimed which include antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative. Moreover, it is also used for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism and scabies. However, it is commonly consumed as vegetable belonging to family *Cucurbitaceae*. Its fruit juice is carminative, tonic, stomachic, stimulant, cardiotonic, enthalmintic, digestive stimulant and also useful in cough, respiratory diseases, fever, intestinal worms, skin diseases and poisonous affections. It improves digestion, calms down sexual urge, caures, anaemia and ulcers. Powdered fruit is useful in healing wounds (Cakici *et al.,* 1994). Extract of fruit pulp, seed, leaves and whole plant of MC has shown hypoglycemic effect in various animal models (Sarkar *et al.,* 1996; Jayasooriya *et al* 2000; Pari *et al.,* 2001; Rathi *et al.,* 2002; Kar *et al.,* 2003; Kiley *et al.,* 1998; Ahmed *et al.,* 2001; Sitasawad *et al* 2000; Grover *et al* 2002; Miura *et al.,* 2001).

Eugenia jambolana

Eugenia jambolana (EJ) commonly known as *Jaman* belongs to the family of *Myrtaceae* is a very common tree of Indian subcontinent. Its bark is astringent and considered specific for dysentery and in preparation of astringent decoction for gargles. Juice of ripe fruit made into vinegar is used as a stomachic, carminative and diuretic. Seeds are used in diabetes treatment (Sastri and Venkataraman, 1962).

Lawsonia inermis

Lawsonia alba Lam. (synonyms *Lawsonia inermis*) (LI) commonly known as *henna* is a perennial plant of the family *Lythraceae, henna* leaves (mehendi) are very popular natural dye to color hand, finger, nails and hair (Joshi 2000). As a medicinal plant, henna has been used for astringent, anti hemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive and sedative effects and used as a folk remedy against amoebiasis, headache, jaundice and leprosy (Sastri 1952). It contains mannite, tannic acid, mucilage, gallic acid and naphtaquinone. On the other hand, its alcoholic extracts possess antibacterial activity against a wide variety of organisms.

Morus alba

Morus alba (synonyms *Morus alba Linn*) (MA) known as white mulberry, is a shrub or moderate sized tree of the

family *Moraceae*. The root bark of mulberry tree has been used by human beings for at least 4000 years as anthelmintic, astringent and purgative. Leaves are effective in diabetes (Joshi 2000), its decoction is used as gargle in inflammation of vocal cords. Fruit is aromatic, laxative and allay thirst in fevers (Sastri 1952). Alkaloids of this plant are known to possess glycosidase inhibitory activity. Chronic subcutaneous administration of the extract of the leaves of MA to rabbits led to degranulation of beta-cells of the langerhans islets (Gulubova and Boiadzhiev, 1975). Increase in glucose uptake was postulated as the mechanism of hypoglycemic action (Chen *et al* 1995). The content and compound of free sugar were determined with plant extracts samples that have been reported to possess significant blood glucose lowering activity.

Nigella sativa

Nigella sativa Linn. (NS), known as black cumin and kalonji is a small medicinal herb of the family *Ranunculaceae*. Its different parts have been reported as therapeutic agents in traditional system of medicines. The seeds of NS are considered carminative, stimulant, diuretic and used in the treatment of mild cases of puerperal fever; they are externally applied for eruptions of skin. Alcoholic extracts of the seeds show antibacterial activity. Seeds yield an essential oil which can possibly be used in cough and bronchial asthma (Deshaprabhu 1966; Dymock *et al.,* 1890; Kirtikar and Basu, 2000). Scientists have found that black seed stimulates bone marrow and immune cell formation, protects the body against viruses, destroys tumor cells and inhibits infection. A plant mixture containing extracts of NS possess blood glucose lowering effects but the direct antidiabetic effect of NS is not yet established. *Nigella sativa* oil (NSO) significantly lowered blood glucose concentration in diabetic rats after 2, 4 and 6 weeks which indicate that the hypoglycemic effect of NSO may be mediated by extra pancreatic actions rather than by stimulated insulin release (EI-DaKhakhny, 2002).

Trigonella foenum graecum

Trigonella foenum graecum Linn. (TF) commonly known as methi belongs to the family *Leguminosae*, used as food and for medicinal purposes (Chadha, 1976). It is a good source of many essential elements such as iron, phosphorus and sulfur (Phillips & Foy 1990); seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect (Chevallier 1996). It is also used in the treatment of late-onset diabetes, poor digestion (especially in convalescence), insufficient lactation, painful menstruation, labour pains etc. (Chadha 1976; Bown 1995). Compounds extracted from the plant have been shown cardiotonic, hypoglycaemic, diuretic, antiphlogistic, hypotensive activity and hypocholesterolemic properties. It may increase plasma insulin level *in vivo* (Gomez and Bhaskar 1998; Khatir 1999). Its major free amino acid 4- hydroxyisoleucine stimulates insulin secretion from perfused pancrease *in vitro* (Al-Habbori and Raman, 1998).

EXPERIMENTAL

Materials

The plant materials were collected from the natural habitat of various localities of Karachi. Glucose Oxidase-Peroxidase kit was purchased from Bio Science, Barcelona Spain. All other chemicals used were of analytical grade.

Preparation of extracts

Dried leaves, fruits and seeds of each plant were pulverized, soaked in methanol (95%) for one week and extracted twice. The extracts were concentrated under vacuum on a rotary evaporator, freeze-dried and stored at -20^{o}C until used. Approximately 1 gm of each extract was transfered in 25 mL volumetric flask and the volume madeup with methanol.

Preparation of calibration curve

Stock solution of 500 mg/l00 mL glucose was prepared in distilled water. From this stock solution, dilutions in the concentration range 60, 80, 100, 120, 140 mg/l00 mL were prepared. For acidic medium all these solution were made in 0.1 M hydrochloric acid and for basic medium buffer of pH 9 was used. Double distilled and deionized water was used for solutions at pH 7. Results given are average of six experiments. The linearity of this method was statistically confirmed (graph 1). Linear regression analyses for the calibration curve (slope, intercept and correlation coefficients) are given in table 1.

Procedure for glucose oxidase method

The assay was performed according to the method described by Khan *et al* (Khan 2005). 0.1 mL of different plant extracts were taken in 10 test tubes. I mL of 100 mg/l00 mL of standard glucose solution and 4 mL of glucose oxidase enzyme was then added in these extracts and kept in dark at room temperature for 2 hours. The calibrated spectrophotometer was adjusted at 506 nm wavelength and absorbance was recorded. The concentration of glucose in the test solution was calculated by the formula,

Uptake of glucose= (Amount of glucose present + added amount of glucose)-glucose left

RESULTS AND DISCUSSION

The results of the present study are summarized in table 2. All plants showed varying effect on glucose utilization. In modern medicine, plants are a potential source of many drugs but still herbs as medicines have not gained enough momentum in scientific community. The aim of this study was to asses the antihyperglycemic effect of six plants

Table 1: Linear regression analysis of proposed method

PH	Slope	Intercept	Correlation coefficient
Acidic	0.0065	0.0488	0.9988
Basic	0.0063	0.0350	0.9972
Neutral	0.0062	0.0422	0.9969

Table 2: Glucose uptake by various plant extracts

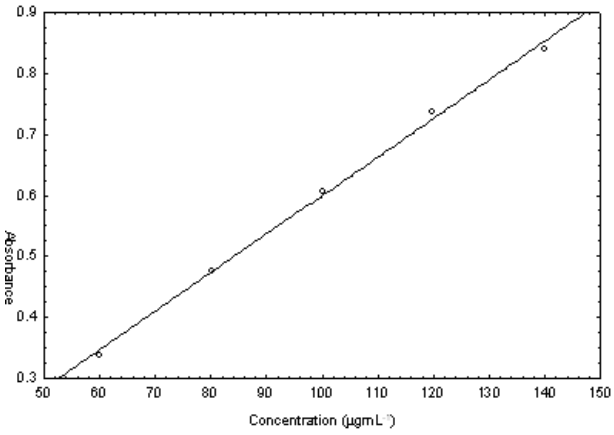
Plant	<—— pH 1 ——>		<—— pH 7 ——>		<—— pH 9 ——>	
	1st Ext*	2nd Ext	1st Ext	2nd Ext	1st Ext	2nd Ext
Nigella sativa seed	49.85	105.62	98.97	0.00	117.62	28.31
Momordica charantia seeds	38.10	343.97	75.93	0.00	189.33	34.36
Momordica charantia pulp	32.88	200.51	4.85	1.26	120.85	40.55
Morus alba fruite	164.59	837.39	91.70	2.09	53.99	145.68
Morus alba leaves	28.52	224.97	62.73	100.00	147.97	36.66
Lawsonia inermis leave	101.27	375.18	57.38	132.00	127.01	83.76
Eugenia jambolana	202.89	669.35	106.37	12.47	397.61	129.69
Eugenia jambolana seeds	113.42	800.51	93.63	27.80	121.41	89.73
Trigonella foenum graecum leave	17.02	126.60	20.78	20.73	0.00	25.07
Trigonella foenum graecum seed	32.67	115.65	27.31	10.88	0.00	33.88

*Extract

and to compare them with the results previously obtained. The results of this investigation revealed that many of these plants have an antihyperglycemic effect. These plants caused a significant decrease in glucose concentration during the experiment.

NS shows significant reduction in glucose concentration in all medium. Literature survey revealed that oral administration of ethanolic extract of NS seeds to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (TBARS and hydroperoxides) and because of its antioxidant effects, its administration may be useful in controlling the diabetic complications in experimental diabetic rats (Kaleem 2006). Fararh (2004) investigated the possible immunopotentiating effect of NS oil on peritoneal macrophages. Hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in treated hamsters after 6 weeks induction of diabetes. Treatment with NS oil significantly increased the phagocytic activity and phagocytic index of peritoneal macrophages and lymphocyte count in peripheral blood compared with untreated diabetic hamsters, this was due to decrease in hepatic gluconeogenesis and the immunopotentiating effect of NS oil was mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes.

MC has enhanced number of beta cells shown to act like insulin or promote insulin release. Drinking of the aqueous homogenized suspension of the vegetable pulp of MC led to significant reduction of both fasting and post-prandial serum glucose levels (Grover and Yadav, 2004). In our study MC seeds show reduction in glucose concentration in neutral medium but no significant reduction was observed at other pH. MC pulp shows approximately the same result.



Graph 1: Linearity in glucose determination by proposed method.

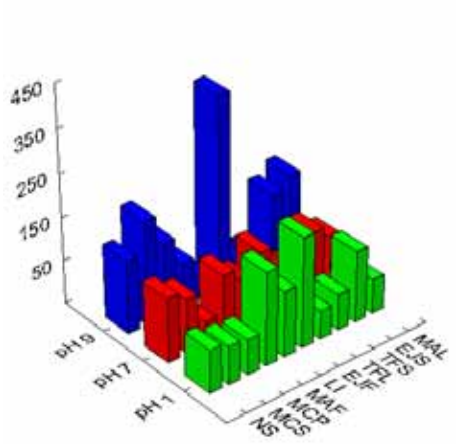


Fig. 2: Glucose uptake activity of plant extracts at various pH levels.

Like MC, MA also acts on beta cell. Gulubova and Boiadzhiev (1975) observed manifested morphologic changes after subcutaneous administration of extract of leaves of MA under the conditions of a chronic experiment. There was degranulation of beta-cells of the langerhans islets of animals developed at various degrees and discovered by means of a light and electron microscope. In our study MA shows reduction of glucose concentration in neutral and basic medium but no change observed in acidic medium.

In the pilot study (mild diabetes), Grover *et al.* (2000) observed maximum reduction of 73.51% in glucose levels in animals receiving 200 mg/kg per day of lyophilized powder of EJ after 3 weeks of treatment. There percent reduction in glucose decreased significantly in the moderate and severe diabetes; 55.62 and 17.72%. The alteration in hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphorfructokinase levels in diabetic mice were partially restored by Grover *et al.* (2000). In *in vitro* study EJ showed minor reduction in glucose concentration in acidic and basic medium but significant reduction in neutral medium.

TF seed and leaves have high hypoglycemic effect in our study. Alarcon-Aguilara *et al.* (1998) also suggested that TF can improve peripheral glucose utilization which contributes to an improvement in glucose tolerance. Moreover till now there is no pharmacological agent that can control diabetic complications.

CONCLUSION

The present study clearly indicated the use of these plants as antidiabetic agents, for example, these herbs can be used as mono therapy or add-on therapy in diabetes management. Further more; their indiscriminate use by the patients may lead to possibility of hypoglycemia.

We believe that these plants may play vital role in future studies on determining the mechanisms of their hypoglycemic activity, as well as for the isolation and identification of active hypoglycemic substances. In addition, further comprehensive pharmacological investigations will be carried out to assess the likely toxicological effects of these antidiabetic plants.

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