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PIPER BETLE LINN: AS A REMEDY FOR DIABETES MELLITUS

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ABSTRACT
Present study was carried out to evaluate the possibility of *Piper betle* Linn. (F: Piperaceae) to be used as a neutraceutical for Diabetes Mellitus. Newly diagnosed, Type 2 diabetes patients from either sex were selected (n=50/group) after measuring fasting blood glucose levels. Either *P. betle* or triphala (a known antidiabetic herbal drug) were given to patients for 30 consecutive days. Treated subjects were checked for fasting blood glucose levels and serum creatinine, urea, aspartate transaminase (AST), alanine transaminase (ALT) levels and selected hematological parameters. According to the results blood glucose levels of *P. betle* treated patients were significantly reduced by 22% and 25% at the end of 2nd and 4th week of the treatment while the blood glucose levels of triphala treated patients were significantly reduced by 14% and 24% at the end of 2nd and 4th week of the treatment compared to their respective pre-treatments. There were no toxic effects as judged by hepatotoxicity, renotoxicity and hematological parameters in both groups. In conclusion, *P. betle* can be used as a potential pharmaceutical for Type 2 diabetic patients.
KEY WORDS: *Piper betle*, Diabetes mellitus, Blood glucose, Toxicity

INTRODUCTION

Piper betle Linn. (F: Piperaceae; Eng: Betel) is a perennial dioecious, semiwoody climber. *P. betle* is cultivated in Sri Lanka, India, Malaysia, Indonesia, Philippine Islands and East Africa. Betel leaves have a strong pungent flavor and is widely used as a masticatory. Betel juice is given to children for cough and administered to the eye for night blindness in adults. It is used to treat catarrh and diphtheria. The leaves are given for gastric and lung disorders in children and applied to purulent ulcers.[1] Experimentally, Sri Lankan grown *P. betle* was shown to have effects on washed human spermatozoa[2], antiaphrodisiac activity[3], antifertility on male rats[4], antimicrobial activity[5], antidiabetic activity[6], antinociceptive activity[7] antioxidant activity[8] and wound healing properties.[9]

Diabetes Mellitus (DM) is a chronic metabolic disorder affecting approximately 4% population worldwide and is expected to increase by 5.4% in 2025. 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 parts of the body system, particularly the blood vessels and nerves.[10] Several botanical neutraceuticals/supplements have been studied as potential therapeutic agents in the management of diabetes and its related complications.

Further, there is a trend to manufacture tablets and capsules from spray dried powders of bioactive plant extracts. Examples include Turmeric spray dried powder, Noni spray dried powder, *Aleo vera* spray dried powder etc. In Asian countries people chew betel leaves.[1] In a previous study, we have shown the antidiabetic activity of *P. betle* hot water extract.[6] This was the first scientific report in the world on antidiabetic activity of *P. betle* in rats. However, no clinical trial was performed using *P. betle* to evaluate its antidiabetic activity in human beings. Therefore, an attempt is made to clinically investigate the antidiabetic activity of *P. betle.* The antidiabetic activity was compared with the well known botanical drug Triphala, which is given to Type 2 diabetic patients. Triphala is a polyherbal preparation which consists of powders of 3 plants [equal proportions (1:1:1) of pericarp of dry fruits of *Terminalia chebula* Retz (F. Combretaceae), *Terminalia belerica* Rox (F. Combretaceae), and *Phyllanthus emblica* Linn (F. Euphorbiaceae)].

MATERIALS AND METHODS

Plant material

P. betle leaves were purchased from main vegetable markets in Western province of Sri Lanka during the period of September - October. The leaves were identified and authenticated by Mr. P.D.S. Wijeratne, the Curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (PS 01) was deposited in the Industrial Technology Institute, Colombo 7, Sri Lanka.

Preparation of *Piper betle* spray dried powder

P. betle leaves were air dried for 3 – 5 days in the shade, cut into small pieces and 20 kg were boiled in 100 L of distilled water for 4 h. The extract was filtered and filtrate was concentrated under vacuum until the total solid content exceeded 25%. The concentrated extract was further subjected to a spray dryer (Kestner Patent Spray Dryer, made in UK; temperature of inlet: 175 ^{o}C and outlet: 100 ^{o}C) to obtain the extract in powder form. The spray dried powder of *P. betle* hot water extract was standardized by (a) determination of physico-chemical parameters, (b) screening phytochemical constituents (c) development of HPLC fingerprints and densitograms and (d) determination of presence/absence of microbes and heavy metals.[11]

Dosage and preparation of *Piper betle* capsules

A dose of 200 mg/kg of *P. betle* hot water extract was found to be the dose that showed best antidiabetic activity in rats.[6] Therefore, in this study we used 2.5 g from the spray dried powder for an adult human (weight: approx. 60 kg) because this dose corresponded to a dose of 200 mg/kg administered to a rat as calculated on the basis of relative surface areas of humans and rats.[12]

Spray dried powder of *P. betle* hot water extract was packed mechanically into capsules (approx. 420 mg/capsule) and used as the test drug. Two capsules (approx. 840 mg) were given to each patient in the "betel group" at 8 h intervals (after 3 meals: breakfast, lunch and dinner) for 30 consecutive days.

Dosage of triphala tablets

Triphala tablets were purchased from Sri Lanka Ayurveda Drug Cooperation, Navinna, Maharagama, Sri Lanka. The strength of a tablet was approx. 500 mg and 2 tablets were given to each patient in the triphala group at 8 h intervals (after 3 meals: breakfast, lunch and dinner) for 30 consecutive days. This is the general dosage of

"triphala tablets" used by Ayurveda physicians to treat Type 2 diabetic patients.

Ethical issues

A clinical protocol was set and was approved (Reg. No. DFAP/2006/01) by the Ayurveda Research Committee, Department of Ayurveda, Sri Lanka. Experiments were conducted in accordance with the internationally accepted ethical guide lines and rules of the ethical committee, Ayurveda Research Committee, Department of Ayurveda, Sri Lanka.[13] This study was performed under the supervision of Ayurveda physicians and written consent was obtained from the patients.

Study participants

One hundred of Type 2 diabetes patients (48 female and 52 males) aged between 35 to 68 years were recruited from 140 responders to news paper advertisements published during the period of May 2007 – December 2009.

The patients who enrolled for the study were allocated into 2 groups and for each patient computer generated code number was given by the Pharmacy. Double blindness was ensured as the participants had no contact with the Pharmacy, that kept the study code and the group of allocation.

Inclusion Criteria

Newly diagnosed type 2 – diabetes patients (previously untreated or blood sugar controlled with diet alone) with fasting plasma glucose level equal to or greater than 140 mg/dL and without any detectable/visible complications were selected. The patients were of either sex (male or female) between the ages of 35 – 68 years. Each subject was interviewed for physical activity and was asked to fill up a 3 day food intake recall form. Subjects with common food intake pattern and physical activity were included in the study. The subjects were managed through dietary consultations and advise.

Exclusion Criteria

Pregnant or nursing patients, patients with hepatic, cardiovascular, renal or endocrine disorder (other than DM) or patients with any complication of DM or patients suffering from Type 1 DM were excluded from the study.

Evaluation of fasting blood glucose levels, liver and kidney functions and hematological parameters

All the treated subjects were checked twice a month (end of 2nd and 4th week) and determined the fasting blood glucose levels. At the end of the study, effects on the liver functions [AST (aspartate transaminase), ALT (alanine transaminase)], kidney functions (urea, creatinine) and hematological parameters [RBC (red blood cell) and WBC (white blood cell) counts, Hb (hemoglobin) concentration] were examined to detect any adverse effects in *P. betle* capsule or triphala tablet treated patients.

Statistical Analysis

Data are given as means ± S E M. Statistical comparisons were made using one way ANOVA followed by Duncans Multiple range test. P value ≤ 0.05 was considered as significant**.**

RESULTS

Base line characteristics of patients

In the group of patients that received *P. betle* capsules, 31 had close family history of diabetes, 27 were male and 23 were female, with mean age of 48.4 ±1.9 years (range 35 – 68), weight 71.4 ± 2.6 kg (range 45 – 76) and a mean duration of known abnormal glucose tolerance of 1.2 ±0.8 years (range 01 month to 5 years). In the group of patients that received triphala tablets, 24 had close family history of diabetes, 25 were female and 25 were male with the mean age of 50.9 ± 2.1 years (range 35 – 68), weight 74.3 ± 1.8 kg (range 45 – 78) and mean duration of known abnormal glucose tolerance of 1.4 ±0.6 years (range 1 month to 5 years). More than 95% patients were none vegetarians in both groups.

Blood glucose levels

After continuous treatment with *P. betle* capsule or triphala tablets significant ($P \leq 0.05$) reduction was obtained in the blood glucose levels of Type 2 diabetic patients (Table 1).

Liver and kidney functions and hematological parameters

As shown in Table 2, all the tested parameters (such as AST, ALT, urea, creatinine levels, RBC, WBC counts and Hb concentration) of *P. betle* capsule or triphala tablets treated patients were within their respective normal ranges.

DISCUSSION

Treatment of hyperglycemia in diabetes involves diet control, exercise and the use of hypoglycemic diets and drugs. However, many oral antidiabetic medicines have a number of serious adverse effects.[14] Herbal drugs are normally used as compound preparations called decoctions, powders and pastes to treat the patients with Type 2 diabetes in Ayurveda and traditional systems of medicine. In modern herbal drug industry, there is a trend to manufacture tablets and capsules from spray dried powders of bioactive decoctions, powders and pastes. The present study revealed a significant ($P \leq$ 0.05) reduction in blood glucose levels of newly diagnosed Type 2 diabetic patients when treated with either capsules filled with spray dried powder of *P. betle* hot water extract or triphala tablets.

The antidiabetic effects of *P. betle* hot water extract and triphala has already been reported in rat models. *P. betle* hot water extract reduced the blood glucose in (a) normoglycemic rats by 24% at 2 h (b) normoglycemic rats by 10% against a glucose challenge (at 3 h) and (c) STZ (streptozotocin) – induced diabetic rats by 33%.[6] The concentrated hot water extract was subjected to a very high temperature (inlet: 175 ^{o}C and outlet: 100 ^{o}C) when preparing the spray dried powder of *P. betle*. However, the antidiabetic effect of spray dried powder of *P. betle* in Type 2 diabetic patients was comparable to that obtained by Arambewela and co – workers[6] in STZ- induced Type 2 rat model. On the other hand, triphala reduced the blood glucose in (a) normoglycemic rats by 17% at 2 h and (b) alloxan – induced diabetic rats by 15%.[15] Similar to results of *P. betle*, antidiabetic effect of triphala tablets in Type -2 diabetic patients was comparable that reported before on alloxan induced Type -2 diabetic rats.[15] Therefore, more or less the antidiabetic effects of *P. betle* capsules and triphala tablets in Type 2 diabetic patients are similar to the results of animal experiments that were carried out using STZ or alloxan induced Type 2 diabetic rat models. In general, it is accepted that, at least a 25 % reduction in blood glucose level should be obtained for drugs used to treat DM. Diabetic patients generally respond to herbal treatment within 3-4 weeks with marked reduction in blood glucose level and little fluctuation throughout the day.[16] However, some patients may require up to 5-8 weeks, respond to a herbal drug. In the present study, *P. betle* capsules significantly ($P \leq 0.05$) and rapidly reduced the blood glucose levels of Type 2 diabetic rats at the end of the 2nd week and this effect was greater than that of triphala tablets. However, at the end of 4th week, the reduction of blood glucose levels in triphala tablets treated group was similar to *P. betle* capsules treated group. Thus, *P. betle* capsules and triphala tablets appear to be suitable to treat Type 2 diabetic patients.

Human diabetics and experimental diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which may result in depletion of the antioxidant defense system and lead to an enhanced de novo free radical generation.[17] In addition, high glucose contents can simply inactivate antioxidant enzymes.[18] Reaction of these free radicals with membrane lipids would result in an increased lipid peroxidation which can be prevented by antioxidants including plant phenolic compounds.[19] Therefore, ability of *P. betle* [8] and triphala [15, 20] to inhibit generation of free radicals may also assist to mediate their antidiabetic effects. *P. betle* capsules and triphala tablets were devoid of unacceptable side -

effects even following sub-chronic administration in humans. There were no overt signs of hepatotoxicity (in terms of AST, ALT), renotoxicity (as judged by serum urea and creatinine) or any adverse effects of hematological parameters (RBC and WBC counts, Hb concentration).

CONCLUSION

This study demonstrated the ability of *P. betle* capsules made out of spray dried powder of betel hot water extract to be used as a potential pharmaceutical for Type 2 diabetic patients.

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Table 1. Effects of *Piper betle* capsules and triphala tablets on blood glucose levels in Type 2 diabetic patients

	Blood glucose levels (mg/dL)		
	Pre-treatment	After 2 weeks	After 4 weeks
Type 2 diabetic patients treated with *P. betle* capsules	182.5 ±5.6	142.8 ±6.4* (22 %)	136.7 ±6.6* (25 %)
Type 2 diabetic patients treated with triphala tablets	192.5 ±9.4	166.2 ±7.9* (14 %)	146.0 ±5.9* (24 %)

Values are expressed as mean ± S.E.M.; n = 50
*Significant when compared with respective pre-treatments: $P \leq 0.05$

Table 2. Effects of *Piper betle* capsules and triphala tablets on liver and kidney functions and hematological parameters in Type 2 diabetic patients

	Type 2 diabetic patients treated with *P. betle* capsules	Type 2 diabetic patients treated with tripala tablets
AST Levels (IU/L)	28.4 ± 1.7 (5 – 40)	28.3 ± 1.9 (5 – 40)
ALT Levels (IU/L)	23.3 ± 2.4 (5 – 30)	26.1 ± 3.7 (5 – 30)
Creatinine (mg/dL)	0.88 ± 0.06 (0.7 – 1.4)	1.0 ± 0.09 (0.7 – 1.4)
Urea (mg/dL)	20.7 ± 1.1 (20 – 40)	18.9 ± 0.09 (20 – 40)
RBC (million/UL)	4.6 ± 0.5 (4.2 – 6.1)	4.4 ± 0.6 (4.2 – 6.1)
WBC (x 10^9 cells/L)	7.8 ±0.3 (4.3 – 10.8)	8.3 ± 0.7 (4.3 – 10.8)
Hb concentration (g/dL)	14.4 ± 0.2 (12 – 16)	13.8 ± 0.3 (12 – 16)

* Figures in parenthesis indicate normal range of each parameter

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