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Research Article

HYPOGLYCEMIC PROPERTY OF ETHNIC VEGETABLES CONSUMED BY THE TRIBAL PEOPLE OF CHITTAGONG HILL TRACTS

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ABSTRACT

The present study reports the hypoglycemic property of some ethnic vegetables which are consumed by the ethnic people of Chittagong Hill Tracts of Bangladesh. It was observed that all of ethnic vegetables, except Miminipata, reduced the diabetic blood glucose level from 9.26mmol/L to 5.04mmol/L over 28 days of treatment. Ethanol extract of Kaminopata was found to be most active. Compared to other extracts, Kaminopata reduced the blood glucose at a faster rate; made the glucose level to 4.76mmol/L (normal limit) within 14 days and sustained it for the rest of treatment period. The next effective ones were Chikipungpata and Khoropata, which reduced the glucose level to respectively 5.39 mmol/L and 5.85mmol/L within 14 days of treatment. The fresh extracts of some vegetables were found to be more effective than the corresponding ethanol extracts. The differences in lowering of blood glucose between the 7 days interval over the period of treatment were found strongly significant ($p<0.05$).

Key words: Hypoglycemic; Ethnic vegetables; Diabetic mice model.

INTRODUCTION

Ethnic people consume almost all of the plant and animal foods, particularly those consumed by birds and monkey; thus they naturally make screening of edible and poisonous foods. The wild plants are an important source of nutrient, vitamin and mineral supplements for indigenous population[1] and also contain a high amount of health promoting phytochemicals, the secondary metabolites with medicinal property. Many epidemiological studies support that a diet rich in fruits and vegetables reduces the risk of incidence of many chronic diseases including diabetes, cardiovascular, neurological, cancers and infections [2]. Foods having medicinal value or health benefit are considered as functional foods[3] and the proper use of functional foods could reduce drug dependency.

From the beginning of the history, nature is the most dependent source of potential drug substances.[4] About 60-80% of world populations still rely on plant based medicines[5] and currently used 25% of modern medicines are made from the medicinal plants first used traditionally[6]. The medicinal action results from secondary metabolites or phytochemicals, the secondary metabolites present in the plants are responsible for the medicinal value and are specific to their taxonomic distinctions[7]. The phytochemicals or bioactive compounds in plants have a wide range of biological functions including antioxidant, antidiabetic, cardioactive, neuroprotective, anticancer, antimicrobial agents[8].

Many drugs which came from folk medicine and traditional use of plants by indigenous communities are currently in the market.[9] Despite discovery of modern medicines, many rural people of Bangladesh still depend on plant products and herbal remedies for treatment of their ailments. Because of its increasing need, some leading pharmaceutical companies in Bangladesh open Herbal and Nutraceticals or Nutricare division along with their Allopathic division.

Diabetes mellitus is a leading health problem worldwide[10]. Its global prevalence was about 8% in 2011 and is predicted to rise to 10% by 2030. Nearly 80% of people with diabetes live in low and middle-income countries[11]. Since long back, plant medicines have been the esteemed source of medicine, thus, they have become an emerging part of high-tech medicine. Several plants or plant products and formulations have been reported to possess anti-diabetic and insulin mimetic or insulin secretory activity[12,13]. Some of the antidiabetic plants are under clinical trials and are found to produce effects on carbohydrate and lipid metabolism when administered as an adjunct on patients with type 2 diabetes [14]. This study investigated the hypoglycemic property of some vegetables consumed by ethnic people of Chittagong Hill Tracts.

MATERIALS AND METHODS

Chemical and Reagent

Alloxan monohydrate was procured from Sigma Chemical Co. (St. Louis, Mo, USA). Hypoglycemic drug glibenclamide- BP 80mg of Square Pharmaceutical was purchased from a drug store. Ethanol was procured from Merck (Dermstadt, Germany).

Sampling Protocol and Sample Collection

On the basis of using as traditional healing agents, seven ethnic vegetables were selected for screening hypoglycemic activity (Table 1, 2). Plant samples were identified and collected from the weekly local markets of Bandarban, Rangamati and Khagrachari districts of Chittagong Hill Tracts. Every variety of vegetables were collected from each of the market locations. The samples were gently water sprayed, packed into fresh poly bags and carried on the same day to the laboratory. These samples were then certified by a Taxonomist of Dhaka University.

Preparation of Crude Extracts
Ethanol Extract

Three samples of every variety of vegetables were pooled together to make a composite sample, from which fresh tender sample was sorted out, washed and rinsed with distilled water. Excess water was wiped out with tissue paper and air-dried. A 500 g of the air-dried composite sample was cut into small pieces, blended in a food processor (Jaipan, JP-FM1100, Jaipan Industries Limited Mumbai, India) and then soaked with ethanol in a ratio of 1:2 for 48 hours. Extracts was collected by filtering through a muslin cloth and then by a filter paper under vacuum. It was then stored into a screw-capped glass vials and stored in a refrigerator.

Fresh Extract

The plant materials were processed as of the ethanol extract. A 500 g of the air-dried composite sample was cut into small pieces and grounded in the food processor. The extract was then collected by filtering through a muslin cloth followed by using a whatman filter paper under vacuum and then refrigerated.

Animal Experimentation

Ninety white albino mice of 25-30 gram weight of both sexes were procured from the animal house of Jahangir Nagar University, Savar, Bangladesh. The animal were housed in stainless cases at standard conditions of 12h light and dark cycle for 7 days at 27^{o}C temperature and were fed with commercial pellet diet with potable water ad libitum. Experiments on animals were performed according to the ethical guidelines of Ethical Committee of the Faculty of Biological Sciences, University of Dhaka.

Screening of Hypoglycemic Activity
Diabetes Induction

Fasting blood glucose level was determined for all of the ninety mice and it was 4.5 (3.5-5.5) mmol/L. In order to induce experimental diabetes, a single dose (120mg/kg body weight) of alloxan monohydrate (Sigma Chemical Co., USA) was injected intra peritoneally to each of the ninety fasting mice. It was found that diabetes was induced in eighty five mice. Five mice did not respond to alloxan. The diabetic manifestation was confirmed by estimating fasting blood glucose level after 72 hours of the alloxan injection; it was characterized by the fasting mean blood glucose level 9.26mmol/L.

Animal Grouping and Treatment

The diabetes mice were divided into 12 groups each comprising 7 mice. Group 1 to10 were used as experimental sets receiving the vegetable extracts, group 11 was used as positive control receiving glibenclamide and group 12 was used as negative control taking only normal diet. The experimental diabetes mice (groups (1-10) were treated with the respective plant extracts (250 mg extract per kg body weight) orally daily for 28 days. The diabetes positive control mice (group 11) received glibenclamide (2.5mg/kg body weight) orally daily for the same period and the diabetes control mice did not give any treatment. All of the mice received pellets diet and water ad libitum everyday.

Glucose Estimation

Fasting blood samples of the mice were drawn by tail bleeding at the time intervals of 0, 7th, 14th, 21st and 28th day for estimation of blood glucose level. Blood samples were collected by tail bleeding into eppendorf tubes and were then centrifuged at 3000 rpm for 15 minutes to separate serum. Serum glucose level was estimated by Glucose oxidase method using commercial kit (Human, Germany) and ELISA plate reader (Labsystem, Finland).

Statistical Analysis

SPSS software package (version 11.5 Inc. Chicago USA) was used to analyze the data. Descriptive statistics were calculated for all variables. Values were expressed as mean±sd. Differences of blood glucose levels between 7 day intervals over the period of 28 days of treatment were performed by independent sample t-test (unpaired ‘t’ test).

RESULTS

Table 2 shows the hypoglycemic activity of the ethnic vegetables consumed by the ethnic people of Chittagong Hill Tracts. The vegetable extracts were indicated to be significantly effective in lowering of glucose level.

It was observed that except Miminipata, all of ethnic vegetables reduced the diabetic blood glucose level from 9.26mmol/L (7.88mmol/L to 10.34mmol/L) to the normal limit 5.04 mmol/L (6.00mmol/L to 4.20mmol/L) over 28 days of treatment. The rate of lowering of glucose level was found to vary with the respect to plant extracts. Ethanol extract of Kaminopata was found to be most active; even it was observed that Kaminopata (at a dose of 250 mg/kg body weight) was found more effective than the therapeutic dose of glibenclamide (2.5 mg/kg body weight). Compared to other extracts, Kaminopata reduced the blood glucose at a faster rate; made the glucose level to 5.97mmol/L) within 7 days, 4.76mmol/L (normal limit) within 14 days and sustained it for the rest of the treatment period. The next effective ones were Chikipungpata and Khoropata, which reduced the glucose level respectively to 5.39 mmol/L and 5.85mmol/L within 14 days of treatment. It was further noted that fresh extracts of some vegetables were found to be more effective than their corresponding ethanol extract such as 4.20 vs 6.04mmol/L, 4.49 vs 5.79mmol/L, 4.28 vs 5.08mmol/L respectively for Ozonshak, Khoropata, Kochi ampata.

Statistical analysis showed that the differences in lowering the blood glucose between the 7 days interval over the period of 28 days of treatment were found strongly significant.

DISCUSSION

Several hypoglycemic agents are being currently used for the treatment of diabetes, but their use have limitations[16,17,18]. Therefore, in search for newer hypoglycemic agent(s), some ethnic leafy vegetables, which are traditionally used in different ailments and so far no scientific study has been done, have screened for their hypoglycemic property on alloxan induced diabetes mice model.

Table 1: Plants tested for hypoglycemic activity[15]

Local name	Scientific name	Edible portion	Traditional use
Kamino	*Caesalpinia digyna* Rottler	Leaves	hypoglycemic, snakebite, rheumatism
Khoropata	*Cissus repens* Lam.	Leaves	perineal healing, retraction of the uterus
Yangfu	*Feics benghalensis* L.	Leaves	diabetic, constipation, antibacterial, costharoag
Ozonshak	*Spilanthes calva* DC.	Leaves	inflammation, toothache, skin diseases, purgative, diuretic, lithotripter and dysentery
Mimini	*Centella assiatica* L.	Leaves	management of central nervous system, skin and GIT disorder
Chikipung	*Rumex vesicarius* L.	Leaves	cooling agent, curing stomach heat, toothache and to check nausea diabetes fever
Kochi Ampata	*Mangifera indica* L.	Leaves	mouth infections, gits disorder, diabetes, diarrhea, scurvy ,typhoid fever, sore throat, dysentery etc.

Table 2: Hypoglycemic Property of Ethnic Vegetables

Plants name	Plant extract and Hypoglycemic agent*	Serum Glucose Level(mmol/L) mean±sd				
		0 day[a]	07th days[b]	14th days[c]	21th days[d]	28th day[e]
Ozon leaves	Ethanol[1]	10.34±0.40	9.32±0.32	8.52±0.48	7.63±0.27	6.04±0.61
	Fresh[2]	9.74±0.55	8.99±0.63	7.24±0.55	6.32±0.34	4.20±o.28
Khoropata	Ethanol [3]	7.88±0.56	6.78±0.27	5.85±0.25	5.76±0.18	5.79±o.11
	Fresh[4]	9.88±0.56	8.28±0.27	7.85±0.25	6.76±0.18	4.49±o.11
Kochi ampatha	Ethanol[5]	9.69±0.49	9.17±0.51	8.75±0.47	6.56±0.42	5.08±0.57
	Fresh[6]	9.17±0.51	7.74±0.53	6.74±0.52	5.10±0.57	4.28±0.32
Chikipungpata	Ethanol[7]	9.42±0.87	7. 37±0.16	5.39±4.17	5.21±4.04	5.42±4.20
Kaminopata	Ethanol[8]	8.19±0.48	5.97±2.96	4.76±2.38	4.76±2.35	4.55±2.26
Miminipata	Ethanol [9]	10.6±0.27	9.82±0.88	10.24±0.62	11.35±0.81	11.24±0.57
Yangfupata	Ethanol [10]	8.03±0.84	7.73±3.70	7.10±3.11	6.67±2.85	5.47±2.69
Positive Control	Glibenclamide[11]	8.85±0.47	7.13±0.32	6.07±0.34	5.19±0.24	4.39±0.32
Diabetic Control	Normal Feed[12]	9.32±023	9.61±0.35	10.18±0.18	10.57±0.25	11.29±0.41

*the numerical number in the superscript indicated animal groupings.

Significance p<0.05 (independent sample t-test)			
1[ab]: t=8.34,p=0.005	1[ac]: t=12.77,p=0.035	1[ad]: t=22.14,p=0.008	1[ae]: t=218.04,p=0.027
2[ab] : t=9.34,p=0.006	2[ac]: t=7.14,p=0.010	2[ad]: t=7.34,p=0.035	2[ae]: t=7.34,p=0.005
3[ab]: t=8.34,p=0.005	3[ac]: t=7.22,p=0.015	3[ad]: t=10.34,p=0.003	3[ae]: t=13.30,p=0.005
4[ab]:t=9.04,p=0.00	4[ac] : t=11.34,p=0.02	4[ad]: t=11.34,p=0.005	4[ae]: t=8.34,p=0.000
5[ab] : t=6.64,p=0.015	5[ac]: t=5.34,p=0.005	5[ad] t=7.74,p=0.005	5[ae] : t=9.34,p=0.008
6[ab]:t=7.34,p=0.015	6[ac] :t=6.34,p=0.005	6[ad] :t=6.34,p=0.00	6[ae] :t=3.34,p=0.000
7[ab]: t=12.34,p=0.005	7[ac]: t =28.34,p=0.015	7[ad]: t=9.34,p=0.025	7[ae]: t=11.30,p=0.000
8[ab]: t=18.30,p=0.008	8[ac]: t=9.33,p=0.050	8[ad]: t=14.34,p=0.015	8[ae]: t=8.34,p=0.003
9[ab]: 0.73,p=2.35	9[ac]: 0.09,p=0.883	9[ad]: 0.79,p=0.973	9[ae]: 0.98,p=1.873
10[ab]: t=8.84,p=0.000	10[ac]: t=9.34,p=0.015	10[ad]: t=11.41,p=0.007	10[ae]: t=9.34,p=0.000
11[ab]:t=3.347,p=0.015	11[ac] t=8.347,p=0.008	11[ad] t=7.347,p=0.005	11[ae] t=9.347,p=0.00
12[ab]:t=0.88,p=0.873	12[ac] :t=0.98,p=0.907	12[ad] :t=0.85,p=0.77	12[ae] :t=0.76,p=1.970

Screening indicated that except Miminipata, single oral dose (250 mg/kg body weight) of the ethnic vegetable extracts reduced the blood glucose level. Lowering of glucose level was found to start from the 7 days and continued over the treatment and made the glucose level normal within the 28 days of treatment period. The extent of glucose lowering was observed to vary with respect to plants and extracts. Some plant (Kaminopata) reduced the glucose level to normal within 14 days and others (Kochi ampatha, Chikipungpata, Khoropata) made it within 21 days, but all of these extracts, except one, lowered glucose level to normal limit within the 28 days of treatment.

Hypoglycemic activity of plants have been reported by a large body of investigators[19,20] and also several plant products are in use in the treatment and management of diabetes[7,8]. However, no one reported hypoglycemic principle of ethnic plants. Phytochemicals in plants are responsible for the medicinal values and their varieties and content make variation in their medicinal property[7,8]. The variation in the hypoglycemic activity of plant extracts tested would be because of different varieties and content phytochemicals in them and also of different mechanisms of hypoglycemic action, like insulin mimetic or insulin secretory activity [7,8] or increase in insulin resistance, suppression of inflammation[21] and reduction or relief of stress by antioxidant activity[22].

The experimental ethnic vegetables have strong hypoglycemic principle. Therefore, the plants can be good candidate to be used as alternative treatment of diabetes. However intensive investigations must be conducted on the fractions of the extracts to identify pharmacological active compound(s), to elucidate mechanism of action and to assure their safety and set appropriate dose. These plants reduce blood glucose and may have beneficial effects on complications of diabetes. Their regular use may help management of hyperglycemia.

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