*O*riginal Research

ScopeMed

Investigation on hypoglycemic effects of ethanol extract of *Alpinia nigra* (Gaertn.) in animal model

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ABSTRACT

Background: Our study aims at exploring the hypoglycemic effect, efficacy, and possible mode of action of ethanol extract of *Alpinia nigra* (EEAN) as an antidiabetic agent in an animal model. **Methods:** Oral glucose tolerance test (OGTT) was used to identify primary hypoglycemic effect in mice. Three tests (glucose absorption, sucrose absorption, and disaccharidase activity) were carried out by gut perfusion and six segments studies to assess carbohydrate absorption and glucose utilization. **Results:** In OGTT, at 400 mg/kg and 800 mg/kg dose of EEAN extract significantly improved oral glucose tolerance among normal mice at 60 min and 90 min with compared to control. Both doses of extract significantly ($P < 0.01$) reduced blood glucose level and showed the hypoglycemic effect by retarding 11.43% and 20.82% of blood glucose level after 2 h of administration in glucose-induced mice, respectively. *In situ* perfused rat intestinal model demonstrated reduced glucose absorption at a 500 mg/kg dose. Inhibition of intestinal disaccharidase was also found by the extract. This was confirmed, yet again, via the six segment study. Throughout the length of the gastrointestinal tract, sucrose digestion was found to be inhibited which is also evident in the six segment study. **Conclusions:** This study suggests that the EEAN has hypoglycemic effects in a dose-dependent manner by inhibiting intestinal glucose absorption, and these may be effective in the treatment of diabetes. Further study is required to explicate the effect this extract or the active compounds have on the individual glucose transporters and the precise mechanism.

INTRODUCTION

Diabetes, a chronic metabolic disorder, is a major threat to global public health that is rapidly getting worse and the biggest impact on an adult of working age in developing countries. There is an estimation of 246 million people with diabetes in the world, of whom about 80% reside in developing countries [1]. Among two types of diabetes, Type 1 causes the immunological destruction of pancreatic β cells resulting in insulin deficiency [2]. Type 2 diabetes mellitus (DM), more prevalent form of the disease, is associated with both impaired insulin secretion and insulin resistance. It is often associated with obesity and hereditary disposition [3]. Multiple lines of therapeutic options have been so far designed and applied to get cure of diabetic ailments. However, the synthetic antidiabetic agents themselves are making numbers of inconveniences due to their side effects along with their higher costs [4]. As a result, alternative therapeutic ways are still in search to shunt those adverse effects caused by synthetic antidiabetic agents.

Traditional preparations from plant sources have recently and widely been used almost every corner of the world as an alternative medication for diabetes due to their less harmful effects and lower prices. The World Health Organization Study Group of DM has also acknowledged the therapeutic advantages of plants medicines in diabetic management as the plants were the first option as antidiabetic therapy before the advent of insulin and oral hypoglycemic drugs this issue [5]. Last two decades, plant materials are progressively formulated and marketed as herbal drugs [6]. It has been estimated that in the U.S. 25% of all prescription dispensed from community pharmacies contain plant extracts [7].

Although the succession of synthetic drugs, to certain extend, has raised the health care of people, until now the use and importance of phytomedicines for the same has never been neglected, and a large number of plants are screened for their efficacy against diabetic and hyperglycemic diseases [8,9]. *Alpinia nigra* (Gaertn.) B.L. Burtt, which belongs to the Zingiberaceae family, is known as Jongly Ada or Tara in Bengali. This aromatic and rhizomatous herb is also referred to as Galangal, False galangal, Greater galangal, Black-Fruited, or Kala. *A. nigra* it is used as traditional medicine for DM. Diabetic patients use it in various forms, e.g. juice of *A. nigra* is a natural cure against DM. *A. nigra*, which is widely cultivated in Asia, Africa, and South America, is a diverse medicinal plant which has also been therapeutically used in the treatment of various diseases. Various therapeutic activities of this plant which has been reported are anti-inflammatory [10], analgesic, antibacterial, cytotoxic [11], anthelmintic [12], anxiolytic-sedative [13], etc. Research showed that isolated compounds from *A. nigra* had well inhibition of α-glucosidase activity [14]. Diabetic patients use it in various forms, e.g. juice of *A. nigra* as a home remedy against DM. The hypoglycemic effect of *A. nigra* was not evaluated by the established methods.

In the present study, we first tried to find out the hypoglycemic effect of *A. nigra* by OGTT. We also tried to establish an indigenous system of medicine (herbal therapy) as antidiabetic drugs instead of chemical drugs. The mode of action of *A. nigra* leaf extract in the treatment of diabetes was also investigated.

MATERIALS AND METHODS

Chemical and Reagents

Reagents of analytical grades and deionized water (Purite, Oxon, UK) were used for the study. Sodium pentobarbital was purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium chloride, D-glucose, sucrose, ethanol, calcium chloride, potassium chloride, and sodium hydrogen carbonate were obtained from BDH Chemical Ltd (Poole, Dorset, UK). All kits were purchased from Boehringer Mannheim GmbH, Germany. Wallac 1409 scintillation counter was supplied by Wallac, Turke, Finland while the microwell plate ELISA reader was obtained from Bio-Tek, USA. Rapid View™ (Blood glucose monitoring system, Model: BIO-M1, BIOUSA Inc, California, USA) with strips were purchased from Anderkilla, Chittagong. Glucose was purchased from the local scientific market, Chowkbazar, Chittagong. Glibenclamide was obtained from Square Pharmaceutical Ltd., Bangladesh.

Collection and Identification

Leaves of *A. nigra* were collected from the Bangladesh Centre for Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh, in the month of April 2014. It was identified and authenticated by the standard taxonomical method at BCSIR.

Preparation of Plant Extract

The collected leaves (5 kg) were washed with fresh water and dried in the shade at room temperature (25°C). The dried leaves were grounded into fine powder by an electrical grinder (Wiley mill) and mesh (mesh number 50) was used to sieve the sample. Then, the powder of leaves of *A. nigra* was pasted by homogenizing with mortar and was suspended with water for preparing the ethanol extract. About 900 g of the leaves were dissolved in absolute ethanol (99% ethanol, source) for 7 days and then filtered. Collected supernatant was dried using a rotary vacuum evaporator (BUCHI Rotavapor R-114). Semisolid crude extracts were again dried with water bath at 80°C. The dried extracts (yield, 12%) were kept in the freezer (4°C) and utilized for biological screening.

Experimental Animals

6-7 weeks old Long-Evans male rats (approximately weighing 110 ± 15 g) and Swiss albino mice were chosen for the study. The animals were bred at BCSIR (Chittagong, Bangladesh). The animals were acclimatized under standard conditions (temperature $23 \pm 2°C$, relative humidity 55%) and were maintained on 12 h light-dark cycle. A standard pellet diet and *ad libitum* were supplied freely unless otherwise indicated. The overall nutrient composition of the diet was 36.2% carbohydrate, 20.9% protein, 4.4% fat, and 38.5% fiber with a metabolisable energy content of 1.18 MJ/100 g (282 Kcal/100 g). The animals were maintained in the laboratory, and the treatment was in the schedule. The animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water.

Hypoglycemic Effect in Glucose-Induced Hyperglycemic Mice

Oral glucose tolerance test (OGTT) was performed according to the standard method [15] with minor modification. Group I was treated as a normal control group, Group II treated with glibenclamide (5 mg/kg body weight), and Groups III and IV were treated with ethanol extract of *A. nigra* leaves at 400 mg/kg and 800 mg/kg body weight, respectively. Glucose solution (1 g/kg body weight) was administered at first. Then, drug and extract solutions were administered to the glucose fed. Serum glucose level of a blood sample from tail vein was estimated using glucometer at 0, 30, 60, 90, and 120 min. Percent decrease of blood glucose level after 120 min measured by the following equation,

$$\% \text{ decrease} = \frac{GL_{0\min} - GL_{120\min}}{GL_{0\min}} \times 100$$

$GL_{0\min}$ = Blood Glucose level at 0 min, $GL_{120\min}$ = Blood Glucose level at 120 min

Sucrose Absorption from Gastrointestinal (GI) Tract

Rats were fasted for 12 h before receiving 50% sucrose solution by gavage (2·5 g/kg body weight) with (for experimental cases) or without (for control cases) ethanolic extract of *A. nigra* (500 mg/kg

body weight). Some of the rats were killed at these timing. The GI tract was excised and divided into six segments: The stomach; the upper 20 cm, middle and lower 20 cm of the small intestine; the cecum; the large intestine. Each segment was washed out with acidified ice-cold saline and centrifuged at 3000 rpm (1000 g) for 10 min. The resulting supernatant was boiled for 2 h to hydrolyze the sucrose followed by neutralization with NaOH. Blood glucose and the amount of glucose liberated from residual sucrose in the GI tract were measured. The GI sucrose content was calculated from the amount of liberated glucose [16].

Intestinal Glucose Absorption

An intestinal perfusion technique [17] was used to study the effect of A. *nigra* on intestinal absorption of glucose in 36 h fasted non-diabetic rats anesthetized using sodium pentobarbital (50 g/kg). The ethanolic extract of A. *nigra* (10 mg/ml, equivalent to 500 mg/kg) suspended in Krebs-Ringer buffer supplemented with glucose (54 g/L) was passed through pyloric, and the perfusate was collected from a catheter inserted at the end of the ileum. The control group was perfused with Krebs-Ringer buffer supplemented with only glucose. Perfusion was carried out at the rate of 0·5 ml/min for 30 min at 37°C, with perfusate being separated by every 5 min. The results were expressed as the percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

Intestinal Disaccharidase Activity

A 20 h fasted rats were killed and the small intestines were isolated, cut longitudinally, rinsed with ice-cold saline and homogenized with 10 ml saline (0·9% NaCl) and centrifuged at 3000 rpm (1000 g) for 3 min. Aliquots (20 μl) of the supernatant from mucosal homogenate were mixed with 1 ml sucrose (40 mmol/L sucrose) in Eppendorf tubes. For the control group, aliquots (20 μl) of distilled water were further added to the Eppendorf tubes. For treatment group, aliquots (20 μl) of A. *nigra* extract of 0.5 mg/ml, 1.0 mg/ml, 2.0 mg/ml, and 5.0 mg/mL were mixed, respectively, in the Eppendorf tubes. These Eppendorf tubes were then incubated with at 37°C for 1 h. Disaccharidase activity was calculated by glucose concentration converted from sucrose as μmol/mg glucose per protein per h [18].

Statistical Analysis

Data were expressed as mean ± standard deviation (SD), $n = 6$ for all experiments. Analyzes were performed by one-way analysis of variance (ANOVA) using statistical software

(Statistical Package for Social Science, version 19.0, IBM corporation NY) followed by Dunnett's *t*-test for comparisons. $P = 0.05$ or less were considered as significant.

RESULTS

Hypoglycemic Effect in Glucose-Induced Hyperglycemic Mice

Investigational induction of hyperglycemia resulted in increased glucose level in blood on mice, which is shown in Table 1. Both doses of leaf extract did not manifest any significant reduction in 30 min after administration. Most significant reduction ($P < 0.05$) was observed for 800 mg/kg dose of ethanol extract of A. *nigra* at 120 min. At 120 min, this dose also showed a significant reduction (20.82%) of blood glucose level. Standard glibenclamide (5 mg/kg) showed a significant reduction in 30, 60, 90, and 120 min, which decrease 40.82% blood glucose level of its initial (0 min). Time interaction with each specific hour in this experiment was also found significant (P < 0.05). Percentage of decrease of blood glucose level in glucose-induced mice after 2 hours with different treatment are also showed in Table 1.

Effects on Sucrose Absorption from GI Tract

Results were expressed as (mean value ± SD) in mg. Administration of extract of A. *nigra* (500 mg/kg) with the sucrose load in rats increased the residual intestinal sucrose content (mg) significantly ($P < 0·05$) at 30 min in the stomach (15.7 ± 2.5*), upper 20 cm small intestine (15.1 ± 2.12*), middle small intestine (21.2 ± 2.86* mg), lower 20 cm small intestine (19.2 ± 2.52* mg), the control rats with the stomach (11.1 ± 2.04 mg), upper 20 cm small intestine (10.2 ± 1.93 mg), middle small intestine (6.5 ± 1.62 mg), lower 20 cm small intestine (3.6 ± 1.21 mg). Residual intestinal sucrose also increased significantly at 60 min in the stomach (7.5 ± 1.86* mg), upper 20 cm small intestine (6.4 ± 1.68* mg), middle small intestine (7.1 ± 1.84* mg), lower 20 cm small intestine (7.8 ± 2.01* mg), the control rats with the stomach (2.1 ± 1.21 mg), upper 20 cm small intestine (1.8 ± 0.76 mg), middle small intestine (2.2 ± 1.32 mg), lower 20 cm small intestine (2.3 ± 1.25 mg). The total sucrose content remaining in the GI tract was increased in A. *nigra* treated rats compared with normal controls [Figure 1].

Effects on Intestinal Glucose Absorption

As shown in Figure 2, intestinal glucose absorption (%) in non-diabetic rats was almost constant during 30 min of perfusion.

Table 1: Effect of *A. nigra* leaves extract on glucose-induced hyperglycemia (mmol/L) in normal mice

Group	0 min	30 min	60 min	90 min	120 min	% decrease of glucose level after 120 min
Control	8.02±2.11	8.37±2.18	7.98±2.11	7.04±1.79	8.29±3.31	-
Glibenclamide (5 mg/kg)	7.3±0.76	6.7±2.06*	5.32±1.67*	4.61±1.18*	4.32±1.01*	40.82
EEAN (400 mg/kg)	6.91±2.25	7.35±1.94*	6.78±1.74*	6.58±2.11*	6.12±2.03*	11.43
EEAN (800 mg/kg)	7.54±1.15	7.86±1.49*	7.21±1.13*	6.67±0.93*	5.97±1.64*	20.82

Values are presented in mean±SD (*n*=6). EEAN=Ethanol extract of *A. nigra* leave. Values with different superscripts in the same column are significantly different from control at each specific hour after the administration of standard and different doses of the extract. For *$P>0.05$ and One-way ANOVA followed by Dunnett's multiple comparison was performed to analyze this comparison, *A. nigra: Alpinia nigra*, SD: Standard deviation
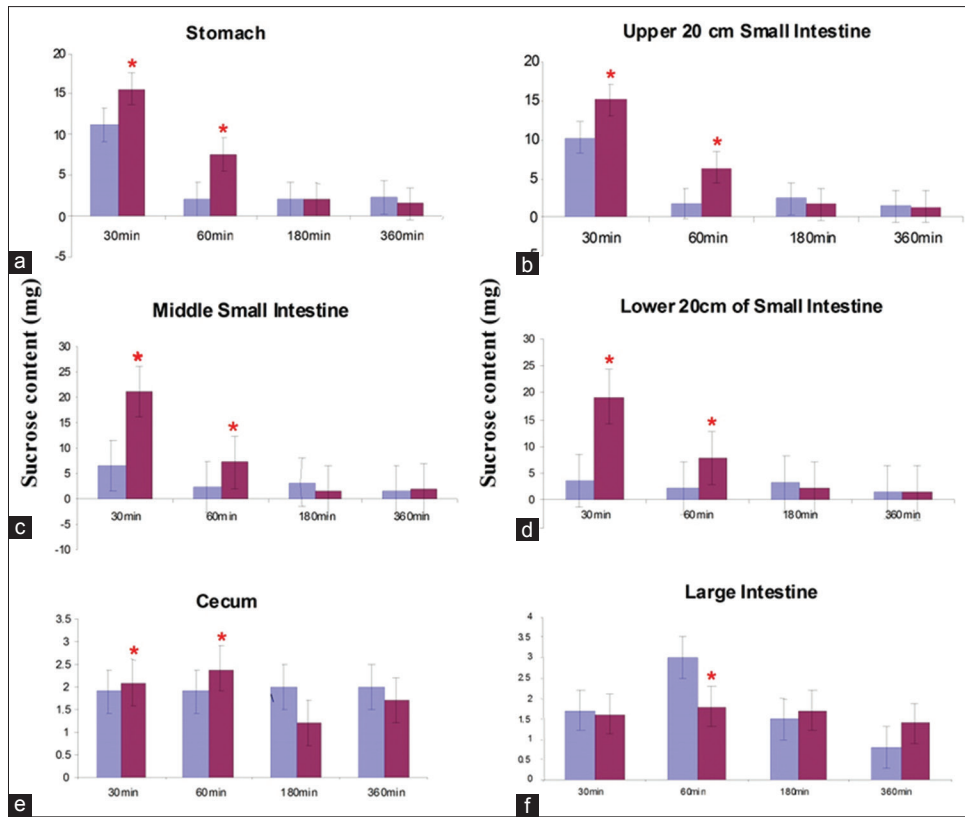
Figure 1: Graph comparing the total sucrose content (a-f) in the whole gastrointestinal tract at 30, 60, 180, and 360 min in a group of control rats versus rats given gavage with *A. nigra* extract. EEAN: Ethanol extract of *A. nigra* leave
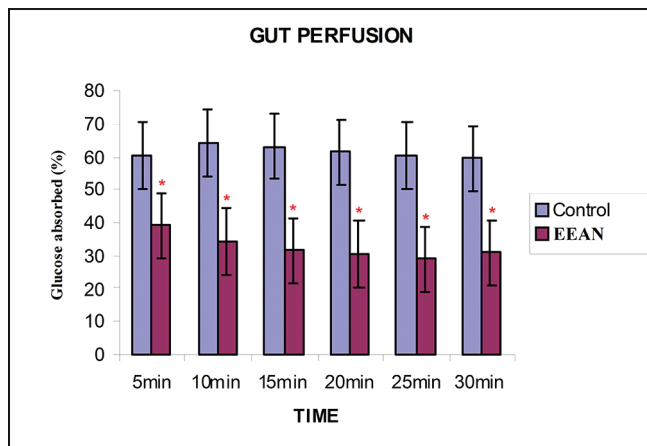

Figure 2: Graph showing the percentage of glucose absorbed in small intestine for 30 min at 5 min intervals. EEAN: Ethanol extract of *A. nigra* leave

The addition of A. *nigra* to the glucose perfusate resulted in a substantial decrease in intestinal glucose absorption during the whole experimental period ($P < 0.05$).

Effects on Intestinal Disaccharidase Activity

Results are expressed in mean ± SD. The intestinal disaccharidase activity decreased significantly in the disaccharide enzymes treated with A. *nigra* at all four concentrations of 0.5 mg/ml
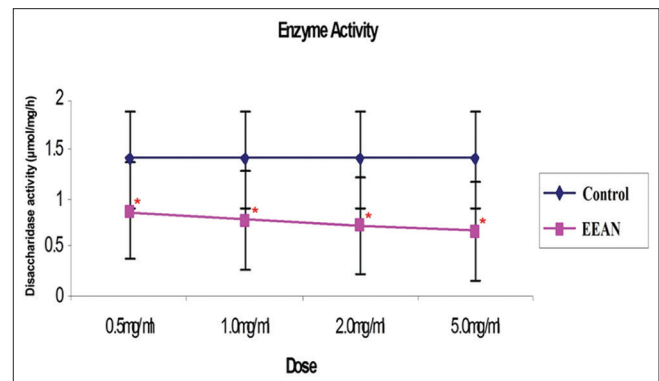

Figure 3: Graph comparing the enzyme activity of the control enzymes extracted from the lumen of rat versus those same enzymes treated with *A.nigra* extract. EEAN: Ethanol extract of *A. nigra* leave

($0.86 \pm 0.06 * \mu$mol/mg/h), 1.0 mg/ml ($0.78 \pm 0.05*$), 2.0 mg/ml ($0.71 \pm 0.05*$), and 5.0 mg/ml ($0.66 \pm 0.07*$), control enzymes (1.39 ± 0.28). The results are depicted in Figure 3.

DISCUSSION

There several tests offered for screening the hypoglycemic result of any sample or drug. However, the OGTT is usually thought of as additional inclined for the screening of impaired glycemia, as a result of it distinguishes the changes in postprandial glycemia that tend to precede changes in abstinence

aldohexose. All the present established diagnostic processes for polygenic disease rely on a threshold price forced on never-ending delivery of blood sugar levels. Yet, the right glycemic threshold that discriminates "normal" from diabetic is not obvious. That's why choice for unknown sort two polygenic disease leftovers a polemic issue, there is clear proof that after it is known, complications are often prevented in several diabetic patients [19,20]. OGTT measures the body's ability to use aldohexose, the body's main supply of energy. OGTT are often accustomed diagnose pre polygenic disease and polygenic disease. The ethanol extract of leaves of A. *nigra* (EEAN) showed vital ability to scale back the elevated aldohexose level in traditional mice compared to the quality drug glibenclamide. At a dose of 800 mg/kg of EEAN showed the highest hypoglycemic effect and it decreased 20.82% of blood glucose level after 2 h of administration in glucose-induced mice, where glibenclamide (5 mg/kg) decreased 40.82%. At dose 400 mg/kg, EEAN decreased 20.82% of blood glucose level after 2 h of administration in glucose-induced mice.

The activity of A. *nigra* extract as antidiabetic drug agent and its doable mechanism was investigated in non-diabetic rats. The post-prandial symptom is undesirable because it will increase glycosylation merchandise, like methylglyoxal, that play a task within the development of diabetic tube disease [21]. Acute elevation of aldohexose conjointly will increase coagulation [22] and leads to multiple disturbances in epithelial tissue cell function [23]. It's renowned that high-fiber diets improve aldohexose tolerance in diabetes [24]. This result could also be attributable to backward stomach removal, increased viscus transit, or modification of the secretion and action of biological process enzymes [25]. The hypoglycemic activity that is found once given with a synchronal aldohexose load in diabetic rats indicates that the extracts could interfere with the viscus aldohexose absorption within the gut by varied mechanisms [26]. In the present study, the various effect of A. *nigra* extract on carbohydrate digestion and absorption in the gut was assessed. This was investigated by gut perfusion experiment where the ethanol extracts showed a gradual decrease in glucose absorption.

Since aldohexose lowering result of genus A. *nigra* was clearly evident from previous study reports, aldohexose absorption inhibition may are a doable mechanism accountable for the hypoglycemic effect [27]. Our study confirms this result similarly as a result of once genus A. *nigra* ethanolic extract was given beside saccharose answer; it considerably increased saccharose retention within the gut compared with solely the saccharose answer au fait cluster of rats. Similar *in vitro* studies dispensed with high concentrations of Glucophage conjointly showed such inhibition of aldohexose absorption [28]. The flavonoids and tannins are reportable to provide antidiabetic activity [29]. This antidiabetic drug property has been connected with the flexibility of the polyphenolic tannins and flavonoids and to inhibit α-glucosidase enzyme [30]. Our study confirmed the claim mentioned higher than since enzyme enzymes of rats treated with A. *nigra* ethanolic extract showed vital dose-dependent inhibition in activity compared with the controls.

CONCLUSION

The present study demonstrates that the ethanol extract of A. *nigra* showed well decrease of blood glucose level after 2 h of administration in glucose-induced mice and significant inhibition of carbohydrate digestion and absorption, which has resulted in the well-known hypoglycemic effects of A. *nigra*. Thus, A. *nigra* may be a useful dietary adjunct for the treatment of diabetes. Further study is necessary to investigate its pancreatic action.

AUTHORS' CONTRIBUTIONS

MSHK and MMNU carried out the study design, data collection, data interpretation, manuscript preparation, statistical analysis, and research grant collection. SMZH collected the plants and participated in experiments, data collection, literature search, and manuscript preparation. MMNU also acted as correspondence. All authors read and approved the final version of the manuscript.

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