EVALUATION OF IN VIVO ANTIDIABETIC AND ANTIBACTERIAL POTENTIAL OF A MANGROVE PLANT- *PANDANUS FOETIDUS*

Farjana Islam Aovi

Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka-1207, Bangladesh.

Abstract: *Pandanus foetidus commonly known as Kewakata is a mangrove plant. This plant has a history of being used among the folk society in diabetic patients in coastal area and for the treatment of various diseases. The present study was aimed to evaluate the anti diabetic and antibacterial potential of P. foetidus. For this study, aerial root parts were used. Antidiabetic activity was assayed against streptozotocin (STZ) induced experimental rats and antibacterial activity was found against 10 bacterial species by disc diffusion method. Streptozotocin induced rats were at first administered to the ethanolic extract. Glibenclamide was used as a standard drug for antidiabetic activity evaluation. Blood glucose levels were determined after oral administration of a dose of P. foetidus (400 mg/kg body. weight) in diabetic groups. Blood glucose levels were determined on 0, 7th, 14th and 21st day after oral administration of the extract (400 mg/kg). The extract was found to reduce blood sugar in streptozotocin induced diabetic rats. Reduction in blood sugar could be seen from 7th day after continuous administration of the extract. The ethanolic extract showed observable antibacterial activity against the bacterial strains Streptococcus pyogens, Salmonella typhi, Escherichia coli & Pseudomonus spp. where Kannamycin was used as standard drug. These results indicated that P. foetidus possess a hypoglycemic and antibacterial effect specially the aerial root parts.*

Keywords: *Pandanus foetidus, Glibenclamide, Hypoglycemia, Antibacterial, Streptozotocin.*

Introduction

In developing countries medicinal plants play an important role in treating many diseases. Diabetes mellitus is a complex disorder that is characterized by hyperglycemia resulting from malfunction in insulin secretion action causing impaired metabolism of carbohydrate, lipids and protein[1]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs[2]. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies[3-8]. More than 400 plant species having hypoglycemic activity have been available in literature[9, 10]; however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect[11]. Besides Diabetes, global prevalence of infectious diseases caused by bacteria is a major public health problem[12, 13]. Within the recent years, infections have increased to a great extent and resistance against antibiotics becomes an ever-increasing therapeutic problem[14].The activities have been selected because of their great medicinal importance. *Pandanus foetidus* Roxb. (Pandanaceae), locally known as kewakata or keora, is a common hedge-plant without a proper stem that grows throughout Bangladesh, predominantly in the coastal mangrove forest region, Sundarban.[15]. Leaves of this plant are used in therapies for leprosy, syphilis, scabies, small pox, and brain, and heart diseases[16, 17]. The leaves and spadix are used to treat

Corresponding author: Farjana Islam Aovi, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka-1207, Bangladesh. Email: farjana.pharm@diu.edu.bd

diabetes[17]. *P. foetidus* has neuropharmacological activity. The root is considered diuretic, depurative and tonic[18]. Therefore, the present study has been carried out to evaluate the antidiabetic and antibacterial activity of *P. foetidus*.

Materials and methods

Plant materials

The aerial root of *P. foetidus* was collected from Koromjal, Mongla, Bagerhat District on 25 November, 2014 and identified by experts at Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen had been submitted (voucher specimen no. 37137 DACB) for future reference.

Test animals

Male Wistar rats of body weight 180–200g were collected from Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. The animals were fed on standard pellet diet (ICDDR, B, Dhaka formulated food) and water ad libitum. The rats used in the present study were maintained in accordance with guidelines of ICDDR, B, Dhaka and the study was approved by the ethical committee of Khulna University.

Test organisms and reagents

The following microorganisms are used as test organisms: *Shigella dysenteriae, Escherichia coli* ATCC 25922, *Salmonella typhi, Vibrio cholera, Pseudomonas aeuruginosa, Pseudomonus spp.* (gram negative bacteria) and *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis, Streptococcus pyogens, Microbispora coralline* (gram positive bacteria).They were obtained from Microbiology Lab of Pharmacy Discipline, Khulna University, Khulna and maintained in Nutrient agar slant at 4^0C for experimental studies.

Preparation of sterile disc

Whatman's No.3 filter paper was punched into 5 mm disc form and they were sterilized, each sterile disc was incorporated individually with 250µg/µl and 500µg/µl of extracts using a micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After 10 minutes another doses of extracts were applied on discs. Then they were stored at 4°C.

Preparation of the root extract

The shade dried aerial root was powdered to get a course granule. About 250 g of dried powder were extracted with 90% ethanol by continuous hot percolation, using Rota vapor apparatus. The resulted dark – brown extract was concentrated up to 100 ml on Rota vapor under reduced pressure. The concentrated crude extracts were lyophilized into powder and used for the study.

The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic extract of *P. foetidus*[19]. Phytochemical screening gave positive test for alkaloids.

Toxicity studies

The animals were divided into six groups separately and were treated orally with ethanolic extracts of *P. foetidus* at 100, 200 and 400 mg/kg, body weight doses.

Streptozotocin-induced diabetic rats

Streptozotocin (STZ) was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intraperitoneal (i.p) injection of streptozotocin at a dose of 50 mg/kg[18]. Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels

determined to confirm diabetes. The rats were divided into 4 groups as follows, first group (Group I) served as normal control, received food and water. Second group (Group II) served as diabetic control, received 0.5 ml of 5% Tween 80; third group (Group III) served as (diabetic control), received glibenclamide (0.5 mg/kg p.o.) and fourth groups (diabetic rats) (Group IV) received 400 mg/kg, body weight of ethanolic extracts of *P. foetidus*. The treatment was continued daily for 21days. Blood drop was collected from the tail for glucose level estimation, just before drug administration on 1st day and 1 h after sample administration on days 7, 14 and 21 (Table 1).

Assay of antimicrobial activity using disc diffusion method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petridishes, after solidification, 100 µl of fresh culture of pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at two concentrations 250 µg/µl and 500 µg/µl. The plates were incubated for 24 hours at 37^0C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) and recorded.

Statistical evaluation

All the data are presented as mean ± SEM. The differences between groups were evaluated by one-way analysis of variance (ANOVA) and <0.01 was considered to be significant.

Results and discussion

Phytochemical screening

Phytochemical screening of the plant ethanolic extract indicated the presence of alkaloids, phytosterols, carbohydrates and saponins.

Toxicity studies

Up to 400 mg/kg body weight was found safe in preliminary test for pharmacological activity in rats. No behavioral or neurological responses on rats were fatal. Acute toxicity studies indicated the non-toxic nature of the ethanolic extracts of *P. foetidus*. The result obtained from the LD_{50} study indicates that ethanolic extract of *P. foetidus* is safer to use in animals even at a dose of 400 mg/kg per oral.

Antidiabetic effects

Effect of ethanolic extract of *P. foetidus* on serum glucose levels in diabetic rats is depicted in Table 1. In animals treated with streptozotocin (50 mg/kg i.p) (Group II), a significant increase in serum glucose level was observed on 7th, 14th, 21st, and 28th day when compared with normal rats (Group I). Group III received glibenclamide (0.5 mg/kg p.o.) showed decrease in serum glucose level when compared with diabetic control rats (Group II). After the oral administration of ethanolic extract of *P. foetidus* in diabetic control rats (Group IV), a significant reduction in blood glucose level was observed on the 7th, 14th, 21st, and 28th day compared with diabetic control rats (Group II).

Anti-bacterial activity

The antibacterial activity of ethanolic extracts of *P. foetidus* aerial roots were investigated using disc diffusion method against selected gram negative bacteria such as *Shigella dysenteriae, Escherichia coli* ATCC 25922, *Salmonella typhi, Vibrio cholera, Pseudomonas aeuruginosa, Pseudomonus spp.* The test was also done against some gram negative bacteria such as *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis, Streptococcus pyogens,* and *Microbispora coralline*. These ten different pathogens have also tested with commercially available antibiotics kanamycin and results were indicated in Table 2. *P. foetidus* ethanolic extract of 500 µg/µl concentration showed higher activity when compared to standard and this may due to the phytoconstiunents present in the extract and in future this kind plant based extract may serve as antibacterial agents to various types of infectious diseases.

Table 1: Anti-hyperglycemic activity of ethanolic extracts of *P. foetidus* on STZ induced diabeticrat

Groups	0 day (mg/ml)	After 7 days (mg/ml)	After 14 days (mg/ml)	After 21 days (mg/ml)	After 28 days (mg/ml)
Group I	60.27±1.45	90.54±1.56	81.58±1.05	78.56±2.79	66.07±2.05
Group II	224.70±15.52	214.5±2.60*	210±4.30*	208.16± 2.38*	201± 16.32*
Group III	233.35±13.9***	182.03±2.8***	130.83±2.20 ***	94.50±5.46 ***	90.2±1.21***
Group IV	237.0 ±15.0***	190.10±3.14***	132.04±1.20 ***	98±10.55 ***	90.04±9.23***

The values are mean ± SEM, n=6, When compared with diabetic control *p < 0.05, **p < 0.001, ***p < 001 (One way ANOVA test)

Table 2: Antibacterial activity of ethanolic extracts of *P. foetidus* aerial roots

Bacterial strains	Type of bacterial strains	Diameter of zone of inhibition in mm		
		Standard Kanamycin) 30 µg	Extract 250 µg	Extract 500 µg
Vibrio cholerae	Gram(-)	29.50	8	12
Shigella dysenteriae	Gram(-)	29.00	5	7.5
Escherichia coli	Gram(-)	25.00	11	14
Pseudomonus spp.	Gram(-)	20.50	6	14
Pseudomonas aeruginosa	Gram(-)	27.00	9	12.5
Salmonella typhi	Gram(-)	28.00	11	18
Staphylococcus epidermidis	Gram(+)	26.50	0	2
Microbispora corallina	Gram(+)	22.00	0	4.5
Streptococcus pyogens	Gram(+)	28.50	7.50	18
Staphylococcus aureus	Gram(+)	30.00	6.5	13

Discussion

Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world and becoming alarming in Bangladesh. Oral administration of ethanolic extract of *P. foetidus* normalized the increased levels of blood glucose in diabetes induced rats. The potent antidiabetic effect of the plant extract suggests the presence of potential antidiabetic active principles, which produced antihyperglycemic effect in diabetic rats[20]. Statistics showed that death from infectious disease had increased more than 50% from 1981 to 1992[21].Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of HIV infections. This situation provided the driving force to the researcher for finding new antimicrobial substances from various source like medicinal plants[22]. In antibacterial assay, the effect was not found as dose dependent. The ethanolic extract showed observable antibacterial activity against the *Streptococcus pyogens, Salmonella typhi, Escherichia coli* & *Pseudomonus spp* except *Staphylococcus epidermidis* & *Microbispora coralline.* The plants of Sundarbans (mangrove forest) have a tendency to possess high levels of tannin as phytoconstituent[23]. Tanin is essential elements of antibacterial agents. So the findings can lead to the development of a new antibacterial molecule.

Conclusion

Oral administration of ethanolic extract of *P. foetidus* reduced the levels of blood glucose level in Streptozotocin induced diabetic rats as compared to control group. Antibacterial activities of the ethanolic extract were evaluated against 10 selected Gram positive and Gram negative bacteria. These studies indicated the presence of active compounds in the ethanolic extract of *P. foetidus* which can be further studied to produce active antidiabetic and antibacterial compounds.

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