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ANTIDIABETIC ACTIVITY OF THE ETHANOLIC EXTRACT OF A SIMPLE ASCIDIAN, *PHALLUSIA NIGRA*

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ABSTRACT

Phallusia nigra is a simple ascidian belonging to the family Ascidiidae occurring as the major component of fouling community on the hull of ships, piers, pilings, harbour installations and materials used for aquaculture operations in the Tuticorin Port Area. The ethanolic extract of *Phallusia nigra* was studied for antidiabetic activity in streptozotocin induced diabetic rats by oral administration of extract (200 mg/kg body weight) for 15 days. The effect was compared with oral dose of 0.5 mg/kg Glibenclamide. Blood glucose levels were determined by GOD-POD kit method. The result shows the ethanolic extract of *Phallusia nigra* significantly lowered the blood glucose of hyperglycemic rats.

KEYWORDS: *Phallusia nigra*, antidiabetic activity, streptozotocin.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%.[2] Management of diabetes without any side effect is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects.[3] Thus searching for a new class of compounds is essential to overcome diabetic problems. There is continuous search for alternative drugs.[4] Ascidians are marine sedentary organisms which ranks second with promising source of drugs.[5] Ascidians or sea squirts are cosmopolitan, exclusively marine invertebrates which

constitute a rich source of biologically active secondary metabolites.[6] Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection.[7] The number of natural products isolated from marine organisms increase rapidly and now exceeds with hundreds of new compounds being discovered every year.[8,9] *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae occurring as the major component of fouling community on the hull of ships, piers, pilings, harbour installations and materials used for aquaculture operations in the Tuticorin Port Area. Previous studies show that the animal possesses antipyretic and analgesic,[10] anti inflammatory,[11] anaesthetic,[12] wound healing [13] and antimicrobial activities.[14, 15] No reports are available on the antidiabetic activity of the simple ascidian *Phallusia nigra*. Hence, the present study focuses on the scientific investigation of antidiabetic activity of the ethanolic extract of *Phallusia nigra*.

MATERIALS AND METHODS

Collection and Identification

Phallusia nigra (Fig.1) was collected from Green Gate area (8˚48'N and 78˚11'E) of Tuticorin Port, Tamil Nadu by SCUBA diving and identified using Key to identification of Indian ascidians.[16] A voucher specimen (AS 2083) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.

Fig. 1: *Phallusia nigra* Sav.

Extraction of the animal material

Epibionts adhering to the test of *Phallusia nigra* were carefully removed, washed several times with sterile sea water, dried under shade and powdered. 100 g of *Phallusia nigra* was

exhaustively extracted with ethanol in a soxhlet apparatus, concentrated in a rotary vacuum evaporator and 15 g of a brown sticky mass was obtained.

Experimental animal

Mature adult Wistar albino rats of either sex weighing about 180 - 200 g were maintained in a well ventilated animal house at 25º± 2ºC and humidity 60± 5% with constant 12 h of darkness and 12 h of light schedule. Clean boiled water and standard pellet diet (Hindustan Lever Ltd., India) were given 'ad libitum'. All the animals were acclimatised to laboratory conditions prior to experiments. 2 ml of 1% Vanillin was used as a flavouring agent to enhance the acceptability of the extract.

Acute oral toxicity studies

To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines.[17] Adult albino rats of either sex weighing 180 - 200 g were used. The animals were divided into six groups of six each. Group I was given 2 ml of 1% saline and Group II received 2 ml of 1% vanillin both acted as control. The other four groups were administered 50, 100, 200 and 500 mg/kgbw of the ethanolic extract with 2 ml of 1% vanillin orally using Intra Gastric Catheter respectively. All the experimental rats were fasted overnight. They were observed continuously for any gross behavioural changes and toxic manifestations like hyperactivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No adverse effect or mortality was detected in this study up to 500 mg/kg bw dose. Hence sub-lethal dose of 200 mg/kg bw doses of the extract were selected for the experiments.

Induction of diabetes

All the rats were fasted overnight before the administration of streptozotocin. Diabetes was induced in rats by intra peritoneal injection of streptozotocin dissolved in 0.1M sodium citrate buffer pH4.5 at the dose of 50mg/kg body weight. After the injection they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycaemic shock. The development of diabetes was confirmed after 48hrs of Streptozotocin injection. The animals having fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation.[18]

Experimental Procedure

In the experiment rats were divided into the following groups with six animals each.

Group I

Normal control received 1% w/v gum acacia 1ml/kg for 15 days orally.

Group II

Diabetic control received 1% w/v gum acacia 1ml/kg for 15 days orally.

Group III

Diabetic rats received ethanolic extract of *Phallusia nigra* 200mg/kg body weight once a day orally for 15 days.

Group IV

Diabetic rats treated with Glibenclamide 0.5mg/kg orally once a day for 15 days. Rats were fasted overnight and the blood was withdrawn from the orbital sinus of the eye on the 5th day, 15th day and 20th day post induction to determine blood glucose by GOD-POD kit method. The change body weight was observed throughout treatment period in experimental animals.

Stastistical Analysis

All values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

RESULTS

The results obtained for antidiabetic studies are presented in Table 1. In the antidiabetic activity, the effects of the ethanolic extract of *Phallusia nigra* was measured on 5th, 15th and 20th day of post induction and were compared with normal and diabetic control groups. Streptozotocin induced diabetic rats showed a significant decrease ($P<0.05$) in body weight compared to normal rats. Oral administration of animal extract at the dose of 200 mg/kg bw showed a significant increase ($P<0.05$) on 15th and 20th day of post induction when compared to untreated diabetic rats. Table- 2 shows the effect of the ethanolic extract of *Phallusia nigra* on blood sugar level in streptozotocin induced diabetic rats.

Table 1: Antidiabetic activity of the ethanol extract of *Phallusia nigra*

Groups	Body weight in gms (Mean±SEM)		
	5th day	15th day	20th day
Group I	157.2±3.25	163±3.54	171±3.34
Group II	153.8±3.34	126.8/±2.10*	115.3±2.39*
Group III	154.3±1.98	160.3±1.764*	165.8±1.47*
Group IV	159.1±2.77	162.8±82.62*	169.5±2.37

Values are expressed as Mean ± S.E. n=6.

P*<0.05 Experimental groups were compared with diabetic control.

P*<0.05 Diabetic groups were compared with control group.

Table 2: Effect of the ethanolic extract of *Phallusia nigra* on blood sugar level in streptozotocin induced diabetic rats

Groups	Blood Glucose Level in mg/dl (Mean±SEM)		
	5th day	15th day	20th day
Group I	61.4±1.22	58.05±1.11	56.47±1.16
Group II	263.16±14.7	251.2±1.34*	248.8±11.88*
Group III	263.10±17.04	125.4±13.99*	60.61±2.24*
Group IV	253.20±3.59	117.06±8.07*	59.06±1.28*

Values are expressed as Mean ± S.E. n=6.

P*<0.05 Experimental groups were compared with diabetic control.

P*<0.05 Diabetic groups were compared with control group.

Oral administration of ethanol extract at the dose of 200mg/kg body weight showed a significant decrease (P<0.05) in blood glucose level in 10 and 15 days of treatment. The fasting blood glucose level on 15th day of post induction (10 days of treatment) was 125.4±13.99* mg/dl compared to group II 251.2±1.34* mg/dl. The group IV treated with Glibenclamide showed fasting blood glucose level of 117.06±8.07* mg/dl. On 20th day of post induction (15days of treatment), the extract treated group showed a fasting blood glucose level of 60.61 ± 2.24 mg/dl, compared to group IV treated with Glibenclamide showed fasting blood glucose level of 59.06± 1.28 mg/dl.

The ethanolic extract of *Phallusia nigra* has exhibited significant antidiabetic property in Streptozotocin induced diabetic rats, as evident from the glucose levels. A preliminary chemical screening of the ethanolic extract of *Phallusia nigra* showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds and tannins. The hypoglycemic activity may be due to the presence of flavonoids, which have shown to inhibit cyclooxygenases and promote β-cell regeneration besides having insulin secretary property.[19,20,21]

CONCLUSION

The results of the present study suggests that the ethanolic extract of *Phallusia nigra* illustrates significant antidiabetic activity, which may be due to the presence of flavonoids in it, as claimed by earlier reports. Further studies on the isolation and structure determination of the active principle and its mode of action are suggested for the development of a new drug.

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