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Research Article

ANTIDIABETIC ACTIVITY OF *APONOGETON NATANS* (LINN.) ENGL. & KRAUSE- AN IMPORTANT FOLKLORE MEDICINE

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ABSTRACT

Objective: The objective of the present investigation was carried out to find the effect of *Aponogeton natans* Linn. leaf with stalks extracts for its antidiabetic activity in different experimental animal models.

Method: The investigation aims on the possible actions of *Aponogeton natans* Linn. leaf with leaf stalks extracts on glucose homeostasis in normoglycemic rats; Oral Glucose Tolerance Test in rats, screening for antidiabetic activity in alloxan induced diabetic rats (single dose and multidose) .

Result: Oral administration of the methanol extract at the doses 200 mg/kg body weight exhibited significant antidiabetic activity in different models. It was observed that methanol extract (ANME) of *Aponogeton natans* Linn. at the dose of 200 mg/kg and Glibenclamide exhibited significant reduction in blood glucose concentration to 84.83±2.23 mg/dl (p≤0.05) and 73.83±1.42 mg/dl (p≤0.01) respectively in comparison to diabetic control group 91.58 mg/dl from 2nd hour. The methanol extract (ANME) (200 mg/kg, p.o.) and standard drug Glibenclamide (2.5 mg/kg, p.o.), significantly decreased the peak blood glucose level to 101.35±9.69 mg/dl and 100.1±9.73 mg/dl (p≤0.05) in comparison to diabetic control group 146.46±9.13 mg/dl after 120th minute of glucose loading. The single administration of methanol extract (ANME) of *Aponogeton natans* Linn. at the test dose level 200 mg/kg p.o. in diabetic rats showed significant (p≤0.01) reduction in blood glucose level respectively. Methanol extract and Glibenclamide (2.5 mg/kg, p.o.) showed significant reduction in blood glucose level to 212.1±4.1 mg/dl and 210.8±4.3 mg/dl (p≤0.01) respectively to diabetic control 312.6 mg/dl after 2nd hours. The results of the study showed that the methanol extract (ANME) possessed significant antidiabetic activity in the multi dose study. Methanol extract (ANME) of and reference standard Glibenclamide (2.5 mg/ kg, p.o.) decreased blood glucose level significant to 166.66±14.00 and 155±14.88 mg/dl (p≤0.01) respectively in comparision to diabetic control 250.5 mg/dl from the 7th day of the experiment.

Conclusion: Hence, present investigation established pharmacological evidences that it can be used as antidiabetic agent. A significant decreases of blood glucose level in single dose multidose study, the methanol extract as compared to control suggests its usefulness in single dose and multidose antidiabetic study.

Keywords: *Aponogeton natans* (Linn.) Engl. & Krause, antidiabetic activity, Glibenclamide, methanol extract.

INTRODUCTION

Aponogeton natans (Linn.) Engl. & Krause.belongs to Aponogetonaceae family. The plant occurs in plains, in the ponds and marshy places in Asia, Australia, India and Srilanka. Leaf pastes are consumed with hot water to treat cuts & wounds [1]**.** Fresh tuber are ground into a paste and boiled with 200 ml of coconut oil and applied on hair before bath for three days to get rid of fungal infection [2]**.** *Aponogeton natans* (Linn.) Engl. & Krause is a important ingredient in preparation of an important Ayurvedic preparation Useerasava. This asava is useful for raktapitta (Haemothermia), anaemia, impurity of blood and diabetes [3]. A perusal of existing reports reveals that the no detailed antidiabetic study had been done earlier. Therefore, the present study has been planned to investigate the antidiabetic activity of various extracts of *Aponogeton natans* (Linn.) Engl. & Krause. leaf with leafstalks in different experimental animal models of diabetes study.

MATERIAL AND METHODS

Materials

Plant material

Fresh parts of *Aponogeton natans* (Linn.) Engl. & Krause.were collected from Salipur, Cuttack, Odisha, India which was identified and authenticated by Prof.P.Jayaraman, PARC, Chennai. The voucher specimen was given the No. PARC/2009/398. The air dried powdered leaves and leafstalks was loaded into soxhlet apparatus and was subjected to extraction for about 72 hours with petroleum ether (60-80ºC), benzene, chloroform and methanol successively. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using rotary evaporator. The extracts were stored in a closed bottle and kept in refrigerator until tested.

Preparation of the test samples

For the pharmacological tests, the extracts were suspended in 3% Tween-80 in normal saline solution to prepare 200 mg/kg concentrations. Glibenclamide (2.5 mg/kg) was used as the reference control. Animals in the control group received only 3%Tween 80 (2ml/kg). All the test samples were administered through oral route.

Drugs and Chemicals

Alloxan monohydrate and Glibenclamide (Sigma-Aldrich Company, St. Louis, Missouri, USA), Accuchek Glucometer kit Accu-Chek Extra Care, Roche Diagnostics India Pvt. Ltd were procured from local market. The solvents and other chemicals were procured from E. Merck, Mumbai and they were of analytical grade quality.

Animals

Wistar albino rats 180-200 g of either sex were used for the experimental models. The animals were obtained from the animal house of Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha. All the rats were kept in standard polypropylene rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed and water given *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee (19/IAEC-IPT/13).

Screening for hypoglycaemic activity using normoglycemic rats

The screening for hypoglycaemic activity using normoglycemic rats was followed as per standard procedures [4]. There was free access to water before and throughout the duration of experiment. The acclimatized animals were fasted for 18 hours. The end of the fasting period was taken as zero time (0 hour), and the collection of blood was done by tail vein punchure of each rat under mild anaesthesia [5]. The blood glucose level was measured with Accu chek Glucometer kit Accu-Chek Extra Care, Roche Diagnostics India Pvt. Ltd. The normal rats were then divided into six groups of six animals in each. Control group was designated as Group I and received vehicle (2 ml/kg) through oral route. Group II, III, IV and V received 200 mg/kg of pet ether, benzene, chloroform and methanol extracts respectively. Group VI received Glibenclamide (2.5 mg/kg p.o.). After 1, 2, 4 and 8 hours of administration of single dose of test sample blood glucose levels were measured.

Screening for oral glucose tolerance test (OGTT) in using rats

The screening for oral glucose tolerance test in rats using rats was followed as per standard procedures [6, 9]. Fasted rats were divided into six groups of six rats in each group. Group I served as a control and received only vehicle (2 ml/kg) through oral route. Group II, III, IV and V received 200 mg/kg of pet ether, benzene, chloroform and methanol extracts respectively. Group VI received Glibenclamide (2.5 mg/kg p.o.). After 30 min of treatment, rats of all groups were loaded orally with glucose (2 g/kg, p.o.). Blood samples were collected before and at 30, 60, 150 and 180 min. after glucose administration as per the method described earlier.

Screening for antidiabetic activity using hyperglycaemic rats (single dose study)

The Screening for antidiabetic activity using hyperglycaemic rats (sigle dose study) was followed as per standard procedures [6, 9]. The acclimatized animals after fasting for 24 hours with water *ad libitum* and then intraperitoneal injection of a dose of 150 mg/kg of alloxan monohydrate in normal saline was given. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anaesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before alloxanisation and 24 hours after alloxanisation. The blood glucose level was measured as stated above. Rats having the blood glucose level above 225 mg/dl were selected for the study [7] and grouped into six groups consisting of six animals each. This condition was observed at the end of 48 hours after alloxanisation. Orally 3% tween 80 solution (2 ml/kg p.o.) was received by the Group I which served as diabetic control, Group II, III, IV and V received 200 mg/kg of pet ether, benzene, chloroform and methanol extracts respectively. Group VI received Glibenclamide (2.5 mg/kg p.o.). After 1, 2, 4 and 8 hours of administration of single dose of test samples, blood glucose levels were measured (Table 3).

Screening for antidiabetic activity using hyperglycaemic rats (multi dose study)

The screening for antidiabetic activity using hyperglycaemic rats (multi dose study) was followed as per standard procedures [8, 9] for this study. The acclimatized animals after fasting for 24 hours with water *ad libitum* received intraperitoneal injection of a dose of 150 mg/kg p.o of alloxan monohydrate in normal saline. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anaesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before and 24 hours after alloxanisation. The blood glucose level was measured as stated above. Rats having the blood glucose level above 225 mg/dl were selected for the study. Then the animals were divided into six groups each containing six animals. Group I served as control, which received only vehicle (2 ml/kg, p.o.), Group II, III, IV and V received 200 mg/kg of pet ether, benzene, chloroform and methanol extracts respectively. Group VI received Glibenclamide (2.5 mg/kg p.o.). The samples under test were administered to the selected animals once daily for 21 days and blood glucose was measured on 1st, 7th, 14th and 21th days respectively.

Statistical analysis

The data obtained from each experiment were subjected to one way ANOVA followed by Dunnet's t test.

RESULTS AND DISCUSSION

Effect of *Aponogeton natans* Linn. extracts on the blood glucose level in screening for hypoglycaemic activity using normoglycemic rats shown in (Fig.1) revealed that the methanol extract exhibited reduction in blood glucose concentration as compared to control. It was observed that methanol extract (ANME) of *Aponogeton natans* Linn. at the dose of 200 mg/kg and Glibenclamide (2.5 mg/kg p.o.) exhibited significant reduction in blood glucose concentration to 84.83±2.38 mg/dl (p≤0.05) and 73.83±1.42 mg/dl (p≤0.01) respectively in comparison to diabetic control group 91.58±2.23 mg/dl from 2nd hour. The chloroform extract, methanol extract and glbenclamide (2.5 mg/kg p.o.) showed significant reduction to 82.21±2.49, 79.87±2.73 and 54.72±54.72 mg/dl (p≤ 0.01) of blood glucose concentration from 8th hour in comparison to control group 92.67±3.22 mg/dl. Whereas the pet ether and benzene extract have not exhibited significant reduction in blood glucose concentration in normoglycemic rats.

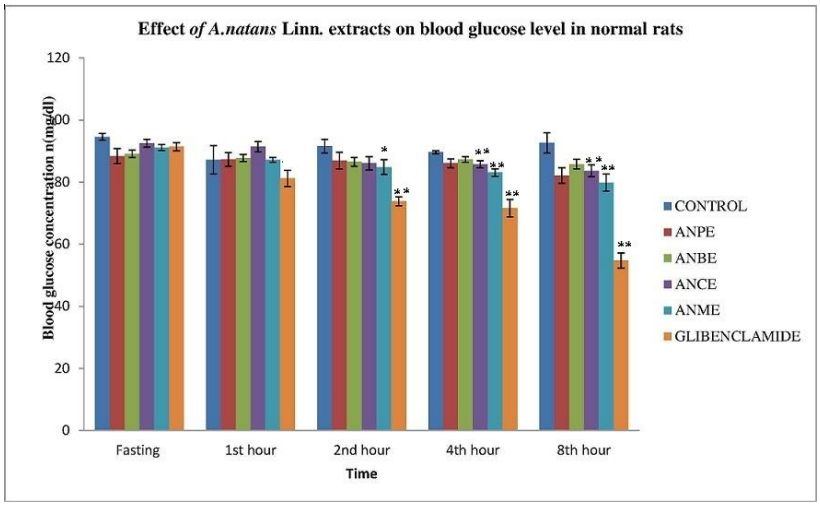

Fig. 1: Effect of *Aponogeton natans* Linn. On blood glucose level in normal rats

ANPE: *Aponogeton natans* pet ether extract; ANBE: *Aponogeton natans* benzene extract; ANCE: *Aponogeton natans* chloroform extract; ANME: *Aponogeton natans* methanol extract. Results expressed as mean ± S.E.M. (n=6).

Significant at $^{*}P<0.05$, $^{**}P<0.01$ as compared with control group (One way, ANOVA followed by Dunnet's t-test).

The effect of *Aponogeton natans* Linn. extracts on oral glucose tolerance test in hyperglycaemic rats is shown in (Fig.2). After 30 min. of glucose administration the blood glucose level increased to peak level and then subsequently decreased. The methanol test extracts exhibited significant hypoglycaemic effect. The methanol extract (ANME) (200 mg/kg, p.o.) and standard drug Glibenclamide (2.5 mg/kg, p.o.), significantly decreased the peak blood glucose level to 101.35±9.69 mg/dl and 101.1±9.73 mg/dl (p≤0.05) in comparison to control group 146.46±9.13 mg/dl after 120th minute of glucose loading. The chloroform extract, methanol extract and standard drug Glibenclamide (2.5 mg/kg p.o.) showed significant decrease of blood glucose level to 102.48±10.5 mg/dl (p≤0.05), 82.53±82.53 and 75.31±6.52 mg/dl (p≤0.01) in 180th minute whereas the pet ether and benzene extracts have not exhibited any significant reduction in blood glucose level during the total period of experiment.

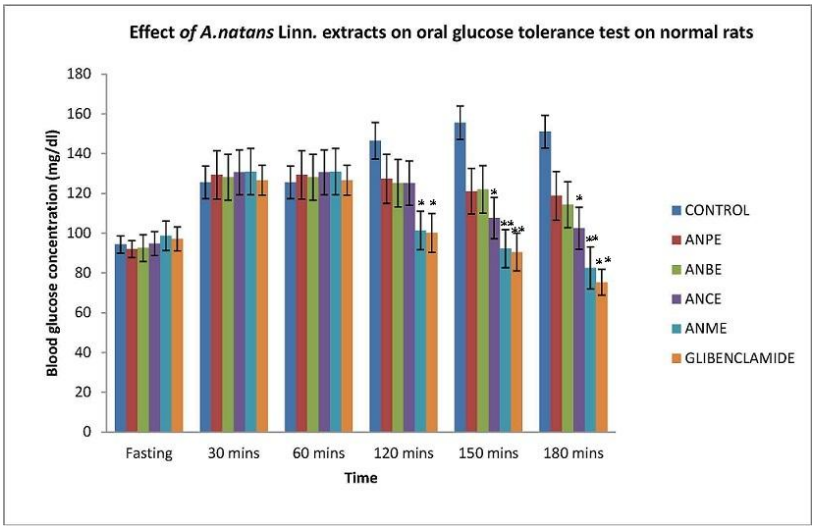

Fig. 2: Effect of *Aponogeton natans* Linn. On oral glucose tolerance test on normal rats

ANPE: *Aponogeton natans* pet ether extract; ANBE: *Aponogeton natans* benzene extract; ANCE: *Aponogeton natans* chloroform extract; ANME: *Aponogeton natans* methanol extract. Results expressed as mean ± S.E.M. (n=6).

Significant at *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test).

In antidiabetic study, the rise in the blood glucose level was observed after 24 hours of alloxanization to the animals. The effect of single dose *Aponogeton natans* Linn. extracts on the blood glucose level in alloxan induced diabetic rats is given in (Fig.3). The single administration of methanol extract (ANME) of *Aponogeton natans* Linn. at the test dose level 200 mg/kg p.o. in diabetic rats showed significant (p≤0.01) reduction in blood glucose level. Methanol extract and Glibenclamide (2.5 mg/kg, p.o.) showed significant reduction in blood glucose level to 212.1±4.1 mg/dl and 210.8±4.3 mg/dl (p≤0.01) respectively to diabetic control 312.6±2.9 mg/dl after 2nd hours. The chloroform extract, methanol extract and Glibenclamide showed significant decrease of blood glucose level to 197.8±4.1, 150.8±2.7, 142.8±1.9 mg/dl (p≤0.01) respectively at 8th hour whereas the pet ether and benzene extract have not exhibited significant reduction in blood glucose level during the total period of experiment.

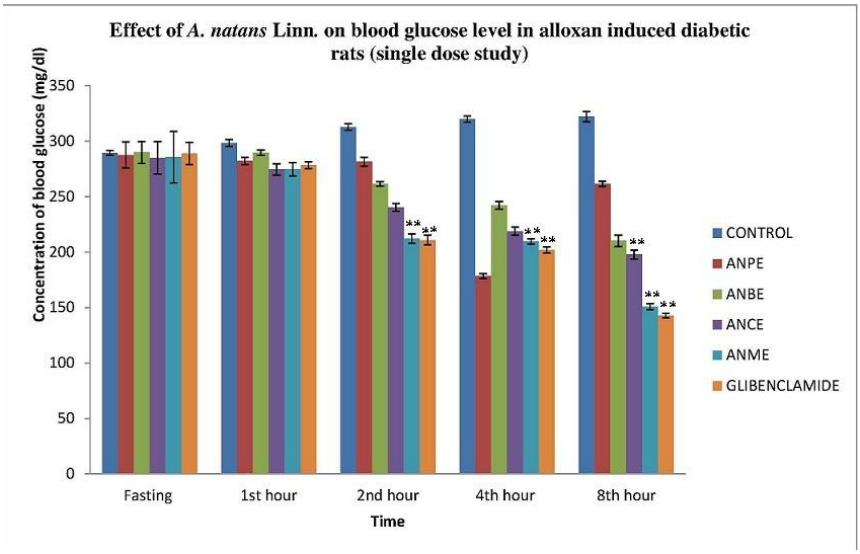

Fig. 3: Effect of *Aponogeton natans* Linn. On blood glucose level in alloxan induced diabetic rats (single dose study)

ANPE: *Aponogeton natans* pet ether extract; ANBE: *Aponogeton natans* benzene extract; ANCE: *Aponogeton natans* chloroform extract; ANME: *Aponogeton natans* methanol extract. Results expressed as mean ± S.E.M. (n=6).

Significant at *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test).

The effect of multi dose *Aponogeton natans* extracts on the blood glucose level in alloxan induced diabetic rats is given in (Fig.4) The results of the study showed that the methanol extract (ANME) possessed significant antidiabetic activity in the multi dose study. Methanol extract (ANME) of the *Aponogeton natans* Linn. and reference standard Glibenclamide (2.5 mg/ kg, p.o.) showed significant activity to 166.66±14.0 mg/dl and 155.00±14.88 mg/dl (p≤0.01) respectively in comparison to diabetic control 250.5±2.71 mg/dl from the 7th day of the experiment. The chloroform extract, methanol extract and Glibenclamide showed decrease of blood glucose level to 202.66±13.68 mg/dl (p≤0.05), 98±9.85 mg/dl and 88.33±9.93 mg/dl (p≤0.01) respectively in comparison to diabetic control group on 21st day whereas the pet ether and benzene extract have not exhibited significant reduction in blood glucose level during the total period of experiment.

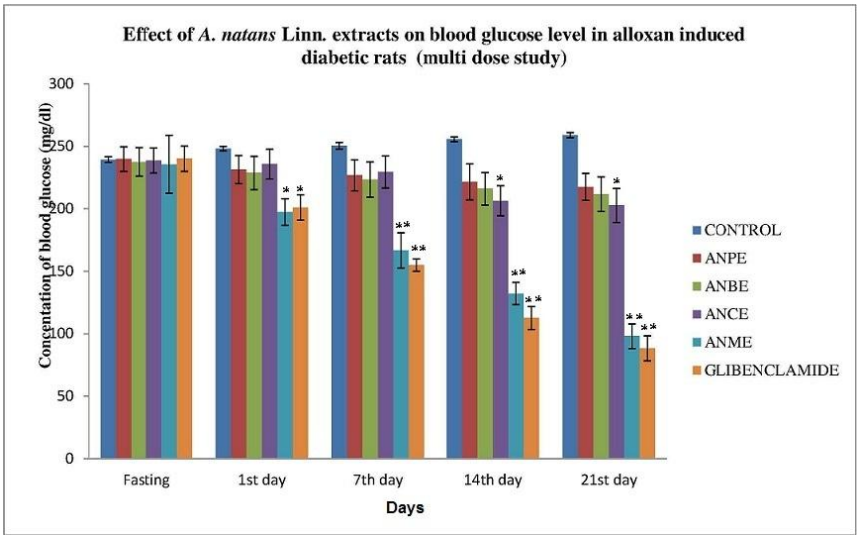

Fig. 4: Effect of *Aponogeton natans* Linn. On blood glucose level in alloxan induced diabetic rats (multi dose study)

ANPE: *Aponogeton natans* pet ether extract; ANBE: *Aponogeton natans* benzene extract; ANCE: *Aponogeton natans* chloroform extract; ANME: *Aponogeton natans* methanol extract. Results expressed as mean ± S.E.M. (n=6). Significant at *P<0.05, **P<0.01 as compared with control group (One way, ANOVA followed by Dunnet's t-test).

CONCLUSION

In conclusion, the result of antidiabetic activity study supports the traditional uses of selected plants as a folk remedy in the treatment of diabetes in the tribal people. The comparable effect of the extracts with Glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic ß-cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extract possesses extra pancreatic effects. Biological active constituents of the extract may be responsible for the said effect.

The results of the present study indicated that, *Aponogeton natans* Linn. methanol extract has significant antidiabetic activity in alloxan induced hyperglycemic rats than pet ether, benzene and chloroform extracts. In comparison with Glibenclamide, it has weaker hypoglycemic activity. The antidiabetic activity of *Aponogeton natans* Linn. methanol extract could be attributed to its constituents like phenolic acids and flavonoids, which possess antioxidant activity as antioxidants have been reported to beneficially improve pancreatic β-cell function by preventing β-cell dysfunction .The results of the present study justify the use of the plant for controlling diabetes as suggested in the folklore remedies.

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