Evaluation of Anti-Hyperglycemic Activity of Adiantum Philippense Linn, a Pteridophyte in Alloxan Induced Diabetic Rats

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Abstract

To evaluate the potential of Adiantum philippense Linn in hyperglycemia induced by alloxan monohydrate, ethanolic and aqueous extracts of the fern, Adiantum philippense were assessed at two dose levels of 500 mg/kg and 250 mg/kg (p.o) respectively. Both the extracts treated groups at two doses exhibited significant hypoglycemic effect when compared to standard drug, glibenclamide and disease control group at an interval of 14 days which is evident from Plasma Glucose levels and Oral Glucose Tolerance Test (OGTT) values.

The serum parameters for kidney functioning like Urea, Creatinine, and lipid profile like Triglycerides, Cholesterol and HDL-cholesterol along with liver enzymes like SGPT and SGOT were assessed which favoured the potential of the fern in causing anti-hyperglycemia. At the end of 14 days study, the levels of liver glycogen of the rats of all the groups were estimated in vitro which showed higher glycogen content in the extract treated groups as compared to the diabetic rats.

To further potentiate the role of this fern in producing hypoglycemia the antioxidant property was evaluated in the extracts through ABTS radical scavenging assay and in the pancreatic tissue by Superoxide Dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH) and Lipid peroxidation (LPO) assays. Among the parameters estimated there was an increase in the levels of SOD, CAT, GSH whereas decrease in the LPO levels in the extracts treated groups which further support the role of Adiantum philippense Linn, in lowering the hyperglycemic state of experimentally induced diabetic rats. Purification of its active principles will be carried out to focus on its lead component that may be helpful in current therapy for treating Diabetes and its complications by rescuing tissues from free radicals and oxidative stress.

Keywords: Adiantum philippense Linn; Hyperglycemia; Alloxan; Liver glycogen

Introduction

Diabetes is a multifactorial disease which has become pandemic nowadays in India and worldwide with the highest population engulfed by this disease causing enormous health problem [1]. The free radicals generated due to oxidative stress pose life-threatening state which is common in hyperglycemic condition [2]. Alloxan monohydrate (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 6, 5-pyrimidinetrione) being a toxic glucose analogue, selectively destroys pancreatic β-cells and causes Diabetes Mellitus in experimental animal [3].

Nowadays, Tolbutamide, Metformin, Gliclazide etc that are highly preferred as anti-diabetic drugs are mainly sulphonylureas and biguanides which are known to cause various adverse effects [4]. Hence in the recent years medicinal plants have become a subject of interest for drug development owing to their phytochemical constituents and their therapeutic potential. In particular, the angiosperms are given more emphasis but the pteridophytes which are significant group of plant kingdom with 12000 species are not much studied for their anti diabetic activity.

Adiantum philippense Linn is commonly known as ‘Hanspadi’ or ‘Walking Maiden hair fern’. It is widely distributed in many parts of India and used as an ornamental plant. It is ethnomedicinally used in bleeding diseases, burning sensation, erysipelas, epileptic fits, dysentery, and elephantiasis [5]. It has been reported that the dried whole plant has been used as a medicine for bronchitis and cough [6]. Adiantum philippense is also reported to have anti-bacterial [7] and anti-fungal activity [8].

Adiantum capillus veneris, a fern of the same genus as that of Adiantum philippense Linn possess antidiabetic potential which is marketed with brand name “Avenca™”. Belonging to the same genus and family of Pteridophytes, insufficient information was available regarding anti diabetic potential of Adiantum philippense Linn (Adiantum lunulatum Burm f.) which led us to investigate its anti-hyperglycemic potential on alloxan induced diabetic rats.

Materials and Methods

Collection and authentication

The fern Adiantum philippense Linn (AP) was collected from ChandraprabhaVanrai, Dapoli in the Ratnagiri district of Maharashtra in the end of monsoon and was authenticated by Dr. P.G Diwakar from Botanical Survey of India, Pune under the number BSI/WC/Tech./2011/306. The animal usage protocol was approved by the Institutional Animal Ethical Committee (IAEC) for National Toxicology Centre through Protocol no.120 on 30/03/2011 and...
executed by following the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals) guidelines.

Preparation of plant extracts

The fronds were cleaned and shade dried in a dryer for 48 hours and percent weight loss was determined. The dried fronds obtained were crushed and kept in the Soxhlet apparatus for obtaining ethanolic and aqueous extract by their respective solvents. The ethanol and aqueous extracts obtained were concentrated in rotary evaporator under vacuum and their percent yields were determined.

Animals

The Male Sprague Dawley rats weighing 200-250 grams were procured from in-house animal facility of National Toxicology Centre, Pune. They were housed under standard conditions of temperature and relative humidity with 12 hr light/dark cycle. Animals were fed on standard commercial pellet diet and water ad libitum.

Acute oral toxicity study

The Acute oral toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organisation for Economic Co-operation and Development) guidelines no 423. Female Swiss Albino mice (18-20 g) were taken for the study and dosed once with 2000 mg/kg of both the extracts. The treated animals were monitored for 14 days [9], for general clinical signs and symptoms as well as mortality. No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/4th and 1/8th doses of 2000 mg/kg i.e. 500 mg/kg and 250 mg/kg were chosen for subsequent experimentation.

Induction of diabetes

Male Sprague Dawley rats were kept on fasting overnight and injected Alloxan monohydrate (Sigma-Aldrich) intraperitoneally (i.p.) for the induction of diabetes at a dose of 150 mg/kg [10]. The animals were bled through the retro orbital plexus and blood was collected in heparinised tube. The plasma glucose was measured in a biochemical analyzer by Glucose oxidase-peroxidase (GOD/POD) method. The rats that developed more than 300 mg/dl of plasma glucose [11] on the 5th day of induction were selected for the study. For treating the diabetic animals with standard reference drug, Glibenclamide was given at a dose of 5 mg/kg orally per day [12].

Animal experimentation

In the present study the animals were distributed into seven groups containing six animals each (n=6) in the following manner:

- **Group 1 (NC):** Normal control
- **Group 2 (DC):** Diabetic control-(Alloxan injected rats);
- **Group 3 (Std):** Diabetic rats+glibenclamide (5 mg/kg);
- **Group 4 (AP 250 eth):** Diabetic rats+250 mg/kg ethanolic extract of AP;
- **Group 5 (AP 500 eth):** Diabetic rats+500 mg/kg ethanolic extract of AP;
- **Group 6 (AP 250 Aq):** Diabetic rats+250 mg/kg aqueous extract of AP;
- **Group 7 (AP 500 Aq):** Diabetic rats+500 mg/kg aqueous extract of AP.

The study was conducted for 14 days to evaluate the potential of the extracts to lower blood glucose level. The body weights of the rats were monitored weekly during the study period.

Biochemical examination

During 14 days study, blood was withdrawn at an interval of 7 days from the retro orbital plexus under anaesthesia of the fasted animals. Blood glucose levels were then estimated by GOD/POD method in an auto analyzer using the commercial enzyme estimation kit (Coral Biosystems, India) besides this, parameters like urea, creatinine, triglycerides, cholesterol, HDL-Cholesterol, SGOT and SGPT were carried out to monitor by commercial kits (Coral Biosystems, India).

Oral glucose tolerance test

The Oral Glucose Tolerance Test (OGTT) was done according to the protocol described by Sheth et al. [13]. In brief, the rats were fasted overnight and the baseline blood glucose was determined next day with the help of an automated glucometer (Accuchekactive	extsuperscript{a}). The rats were loaded with 2 g/kg of glucose 30 minutes after administration of drug at their respective doses. The blood glucose was monitored for 30, 60 and 120 minutes thereafter.

Liver glycogen analysis

The assessment of liver glycogen was undertaken by following the protocol described by Goel et al. [14]. At the end of the 14 days study, animals were dissected out and 1 g of fresh liver tissue was put into weighed tubes. They were loaded with 2 g/kg of glucose 30 minutes after administration of drug at their respective doses. The glucose was monitored for 30, 60 and 120 minutes thereafter.

Lipid Peroxidation (LPO): Lipid peroxidation in the tissue was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) which were homogenized in the incubation medium using a Teflon homogenizer and 1.0 ml of homogenate was mixed with 2.0 ml of TBA-TCA-HCl reagent [20]. The contents were incubated...
in a boiling water bath for 15 minutes and the pink colour developed was estimated at 535 nm against a reagent blank, in a spectrophotometer [21]. The MDA equivalents of the samples were calculated using the extinction coefficient 1.56×10^3 M⁻¹ cm⁻¹.

d. Reduced glutathione (GSH): Reduced glutathione on reaction with DTNB (5,5'-dithiobis-2-nitro benzoic acid) produces a yellow colored product that absorbs at 412 nm [22]. Briefly a homogenate was prepared with 0.1 g of the pancreas with 0.5 ml of 5% TCA. The precipitated protein was centrifuged at 1000 rpm for 10 minutes. The supernatant (0.1 ml) was made up to 1 ml with phosphate buffer and mixed with 2 ml of DTNB for the estimation of GSH. The values were expressed as n moles of GSH/g of tissue [23].

e. Protein analysis in pancreas: Protein concentrations were measured using the method of Lowry et al. [24]. Bovine serum albumin was used as a standard.

Histopathology

At the end of the study animals were dissected out and the organs were fixed in 10% formalin for monitoring necrosis or adverse effect of the treatment. The tissues were processed for histopathology starting from trimming, graded alcohol dehydration, embedding in paraffin, sectioning, spreading, fixing in the slides and staining with hematoxylin and eosin. The slides were seen under the microscopy (200 X) for abnormalities and photomicrographs were taken.

Statistical analysis

The results were analyzed for statistical significance by one way ANOVA and were expressed as Mean ± SD by using Graph pad Prism 5 version (Dunnett’s multiple comparison tests). P<0.001 were considered as statistically significant.

Results

The body weights were assessed at an interval of 7 days. The control animals showed a linear proportion of growth in terms of weight whereas the untreated diabetic rats were found to have significant weight loss of 23.96%. However, administration of AP ethanolic and aqueous extract at two different doses and standard drug (Glibenclamide) to diabetic rats restored the body weight to a normal level (P<0.001; Table 1).

Alloxan induced diabetic rats showed a 74.45% rise in the plasma glucose levels as compared to normal control group. Treating those hyperglycemic rats with two doses of ethanolic and aqueous extract of Adiantum philippense (250 mg/kg and 500 mg/kg) and standard drug (5 mg/kg) resulted in significant reduction in the plasma glucose level (P<0.001) which came at par with the normal control animals at the end of 14 days study. On the other hand the diabetic control animals which received no treatment continued to show high plasma glucose throughout the study (Figure 1).

In alloxan induced diabetic animals, elevated levels of serum urea and creatinine were observed. On treating the animals with both standard and test drugs the elevated levels decreased to almost normal range. However, the untreated diabetic control group showed high levels of serum urea and creatinine till the end (P<0.001; Table 2).

In the present study the lipid profile was assessed by monitoring the parameters like triglycerides, cholesterol and HDL-cholesterol. It was found that hypertriglyceridemia and hypercholesterolemia occurred with the induction of diabetes when compared with the normal control group (P<0.001). Treatment with standard drug and both the extracts of AP at different doses resulted in a reduction of those levels significantly (P<0.001) when compared with the diabetic control group. On the contrary, the HDL-cholesterol level was found to be lower in the beginning but later a significant increase in the levels were observed when supplemented with two extracts of AP and standard drug while the disease control group continued to remain low (P<0.001; Table 3).

SGPT levels were augmented at the induction of diabetes but later on with the administration of plant extracts it was able to lower the SGPT levels which were comparable to that of standard drug but was unable to bring down the levels to normal (P<0.001). Both the ethanol and aqueous extract of AP at dose of 500 mg/kg and 250 mg/kg when

| Groups     | Body weight (in grams) | Initial | Final | % difference |
|------------|------------------------|---------|-------|--------------|
| NC         | 222.00 ± 2.44          | 230.33 ± 4.08 | 3.75↑  |
| DC         | 216.00 ± 2.28          | 171.83 ± 5.84** | 23.96↓ |
| Std        | 225.00 ± 4.73          | 233.16 ± 4.16*** | 3.62↑  |
| AP 250 eth | 221.50 ± 2.86          | 227.66 ± 3.44*** | 2.78↑  |
| AP 500 eth | 222.30 ± 1.40          | 233.83 ± 3.76*** | 3.34↑  |
| AP 500 aq  | 224.33 ± 2.30          | 233.83 ± 4.16*** | 4.70↑  |

Values are expressed as Mean ± SD
NC: Normal Control; DC: Diabetic Control; Std: Standard Drug; AP: Adiantum philippense; aq: Aqueous; eth: Ethanolic Extract
***P<0.001(compared with normal control)
**P<0.01(compared with diabetic control)

| Parameters     | NC      | DC      | Std     | AP 250 eth | AP500 eth | AP 250 aq | AP 500 aq |
|----------------|---------|---------|---------|------------|-----------|-----------|-----------|
| Urea (mg/dl)   | 20.83 ± 1.7 | 61.33 ± 2.7** | 37.17 ± 2.8*** | 37.83 ± 2.2*** | 34.00 ± 2.1*** | 42.17 ± 2.4*** | 39.83 ± 2.4*** |
| Creatinine (mg/dl) | 0.35 ± 0.05 | 1.7 ± 0.21*** | 0.8 ± 0.09*** | 0.70 ± 0.11*** | 0.60 ± 0.09*** | 0.6 ± 0.08*** | 0.67 ± 0.12*** |

Values are expressed as mean ± SD
NC: Normal Control; DC: Diabetic Control; Std: Standard Drug; AP: Adiantum philippense
***P<0.01(compared with normal control)
**P<0.01(compared with diabetic control)

Table 1: Effect of AP on the body weight of the diabetic animals.

Table 2: Effect of AP on serum urea and creatinine of experimental animals.
Table 3: Effect of AP on lipid profile of experimental animals.

| Parameters | NC | DC | Std | AP 250 eth | AP500 eth | AP 250 aq | AP 500 aq |
|------------|----|----|-----|------------|-----------|-----------|-----------|
| Triglycerides (mg/dl) | 85.67 ± 1.4 | 292.5 ± 3.5*** | 74.83 ± 3.3*** | 84.33 ± 3.8*** | 82.33 ± 3.6*** | 86.67 ± 3.4*** | 86.50 ± 3.1*** |
| Cholesterol (mg/dl) | 55.5 ± 3.5 | 92.83 ± 3.4*** | 53.67 ± 2.3*** | 55.17 ± 3.4*** | 53.5 ± 1.8*** | 64.0 ± 3.6*** | 56.17 ± 2.1*** |
| HDL cholesterol (mg/dl) | 32.66 ± 1.4 | 32.1 ± 2.3*** | 33.83 ± 1.7*** | 32.2 ± 1.6*** | 38.7 ± 1.9*** | 29.6 ± 1.9*** | 36 ± 2.3*** |
| Values are expressed as mean ± SD
NC: Normal Control; DC: Diabetic Control; Std: Standard Drug; AP: Adiantum philippense
***P<0.001(compared with diabetic control)
**P<0.01(compared with diabetic control)

Table 4: Effect of AP on liver function of experimental animals.

| Groups | CAT (U/mg of protein) | SOD (U/mg of protein) | GSH (mole/gm of tissue) | MDA (U/mg of protein) |
|--------|-----------------------|-----------------------|-------------------------|-----------------------|
| NC     | 1.88 ± 0.016          | 0.98 ± 0.017          | 25.47 ± 0.752           | 0.36 ± 0.015          |
| DC     | 0.60 ± 0.026***       | 0.59 ± 0.154***       | 19.01 ± 1.227***        | 1.01 ± 0.019***       |
| Std    | 1.29 ± 0.033***       | 0.96 ± 0.136***       | 27.70 ± 0.847***        | 0.53 ± 0.012***       |
| AP250eth | 1.55 ± 0.028***      | 0.95 ± 0.013***       | 23.93 ± 1.324***        | 0.47 ± 0.011***       |
| AP500eth | 1.65 ± 0.031***      | 0.97 ± 0.013***       | 28.75 ± 1.770***        | 0.46 ± 0.011***       |
| AP250 aq | 1.53 ± 0.011***      | 0.76 ± 0.147***       | 22.78 ± 0.736***        | 0.56 ± 0.017***       |
| AP500 aq | 1.59 ± 0.011***      | 0.85 ± 0.156***       | 25.30 ± 0.456***        | 0.52 ± 0.014***       |

Table 5: Effect of Adiantum philippense on pancreatic antioxidant enzymes and lipid peroxidation levels.

| Groups | CAT (U/mg of protein) | SOD (U/mg of protein) | GSH (mole/gm of tissue) | MDA (U/mg of protein) |
|--------|-----------------------|-----------------------|-------------------------|-----------------------|
| NC     | 1.88 ± 0.016          | 0.98 ± 0.017          | 25.47 ± 0.752           | 0.36 ± 0.015          |
| DC     | 0.60 ± 0.026***       | 0.59 ± 0.154***       | 19.01 ± 1.227***        | 1.01 ± 0.019***       |
| Std    | 1.29 ± 0.033***       | 0.96 ± 0.136***       | 27.70 ± 0.847***        | 0.53 ± 0.012***       |
| AP250eth | 1.55 ± 0.028***      | 0.95 ± 0.013***       | 23.93 ± 1.324***        | 0.47 ± 0.011***       |
| AP500eth | 1.65 ± 0.031***      | 0.97 ± 0.013***       | 28.75 ± 1.770***        | 0.46 ± 0.011***       |
| AP250 aq | 1.53 ± 0.011***      | 0.76 ± 0.147***       | 22.78 ± 0.736***        | 0.56 ± 0.017***       |
| AP500 aq | 1.59 ± 0.011***      | 0.85 ± 0.156***       | 25.30 ± 0.456***        | 0.52 ± 0.014***       |

Discussion

Diabetes and its complication goes hand in hand, primarily uncontrolled hyperglycemia followed by decrease in body weight which can be attributed to a phenomenon known as muscle wasting due to rapid catabolism of fat and protein [25]. In the present study the diabetic animals showed 26.96% decrease in body weight whereas the standard and the treated groups gained 3-4% body weight in duration of 14 days.
The condition of hyperglycemia which was prominent in diabetic group of animals was also brought to normal levels with a daily dosing of the two extracts namely ethanolic and aqueous at 500 and 250 mg/kg doses. The ethanolic extract at a dose of 500 mg/kg lowered the elevated plasma glucose level by 66.30% while the same extract at 250 mg/kg was able to lower the plasma glucose by 63.50%. The aqueous extract treated groups at 500 mg/kg and 250 mg/kg showed a reduction of plasma glucose by 61.46% and 58.69% respectively. The reduction percent in case of standard drug treated group was found to be 62.16% and the diabetic control group continued to show a 2.21% increase in their plasma glucose concentration.

Plasma urea and creatinine are believed to be specific markers in diabetes for proper functioning of kidney [26]. Elevated levels of urea and creatinine were observed in diabetic animals owing to the adverse effect of alloxan on kidney [27,28]. These elevated levels came down to a normal level after treating the animals with standard drug and both the extracts of AP at two different doses which was evident from the results.

Induction of Diabetes to animals by alloxan monohydrate often shows elevated triglyceride and cholesterol levels [29] and reduced HDL-cholesterol values [30]. Similarly in this study the lipid parameters were performed where diabetic control animals showed higher triglycerides and cholesterol levels and lower HDL-cholesterol values which when treated with standard drug and test extracts resulted in lowered serum cholesterol and serum triglyceride levels and an increase in the HDL-cholesterol level.

During diabetic condition, high levels of SGPT and SGOT were found in rats [31]. In the present study normal levels of hepatic biomarkers like SGPT and SGOT were obtained after administration of the extracts for 14 days suggesting restoration of proper hepatic function.
Oral Glucose Tolerance Test being a potent diagnostic test during a diabetic study was carried out which showed a rise in the blood glucose level after administering glucose load which later came to the baseline at an interval of two hours but the diabetic group took more time to come to the baseline indicating damage of pancreas caused by alloxan. Moreover, while monitoring the hepatic glycogen concentration, a lower concentration of glycogen was found in the liver of the diabetic animals which can be attributed to the high amount of glucose circulating in their blood due to insulin insufficiency. The situation was however reversed in case of both the extracts treated and standard drug, Glibenclamide treated animals. Similar results were obtained in our previous study which was undertaken with another species of fern namely *Pterisvittata* L. [32] indicating a pivotal role of pteridophytes in diabetic condition.

As put forth by a recent research, free radicals being highly reactive in nature alters cellular morphological and functional integrity, damages membrane proteins leading to cell inactivation and hence body cells develop certain mechanisms to scavenge them either through enzymatic or non-enzymatic means [33].
Thus in the present study Superoxide Dismutase (SOD), Catalase (CAT), and reduced glutathione which are known to be involved in the direct elimination of active oxygen species [34] were evaluated along with lipid peroxidation that is often used as a marker for free radical induced tissue injury [35].

The ABTS radical scavenging activity of the both the extracts showed increasing antioxidant ability at increasing concentrations (Figure 4). In the in vivo study as well, the results obtained suggested that treating the diabetic animals with the two extracts of AP at two doses and standard drug can enhance the levels of SOD, CAT and GSH which otherwise in experimentally induced diabetic rats remains suppressed. LPO being one of the cellular features of severe diabetes [36], its level remain quite high in case of diabetic animals which in the present study reverted to normal in animals receiving the extracts of AP as well as standard drug. The histological observation of the pancreatic tissue from figure 5A shows the islet cells which are normal in their architecture whereas the figure 5B indicates that the Alloxan reduces the number of size of islets in the pancreas of the Diabetic rats. While figure 5C-5G suggests that Standard drug and both the extracts of *Adiantum philippense* might have a protective or regeneration property on pancreatic tissue because larger and prominent islets were seen at the end of 14 days treatment of the diabetic rats.

As an outcome, it can be concluded that the results indicate *Adiantum philippense* Linn possess anti-hyperglycemic as well as antioxidant potential and thus further studies on bioactive or fractions shall be evaluated for control and management of diabetes mellitus.

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