Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2014; 6(4): 341-347

ISSN- 0975 1556

Research Article

# Antidiabetic Potential of Whole Plant of *Adiantum capillus veneris* Linn. in Streptozotocin Induced Diabetic Rats

*Vadi Ranjan, Manisha Vats, Neha Gupta, Satish Sardana

*Department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonepat-131001, Haryana.*

*Available Online: 1th October 2014*

**ABSTRACT**

The present study aims at investigation of the antidiabetic efficacy of aqueous and methanol extracts of whole plant of *Adiantum capillus veneris* Linn. in streptozotocin induced diabetic rats. *Adiantum capillus-veneris* L. (Family Adiantaceae) commonly known as hansraj is a terrestrial fern occurring throughout the India in moist shady places especially on damp old walls and crevices of rocks. Plant leaves and stem were found to contain higher amount of fats, flavonoids, triterpenoids, phenols, tannins, saponins and fats. After 72 h of STZ (45 mg/kg bw, i.p.) administration, animals showing serum glucose level more than 250 mg/dl were considered as diabetic rats. Aqueous extracts at dose of (100, 200 and 400 mg/kg, orally) and methanol extracts at dose of (200 and 400 mg/kg, orally) in distilled water were administered daily for 21 days and metformin (50 mg/kg b.wt.) was taken as the standard. FBG were measured by glucose oxidase method on 0th day (after 72h of STZ), 10th, 21st day. The serum was separated by centrifugation at 4000 rpm for 10 min. Improvement in the FBG indicates that *Adiantum capillus veneris* Linn. has very good antidiabetic potential with very low side effects and provides a scientific rationale for the use as an antidiabetic agent, thus justifying its traditional usage. From the phytochemical analysis, it was found that the major chemical constituents of the extract were flavonoids and tannins. On the basis of the above evidences it is possible that the presence of flavonoids and tannins may be responsible for the observed antidiabetic activity.

**Keywords:** *Adiantum capillus veneris* Linn, Diabetes mellitus, Flavonoids, Fasting blood glucose, Streptozotocin.

## INTRODUCTION

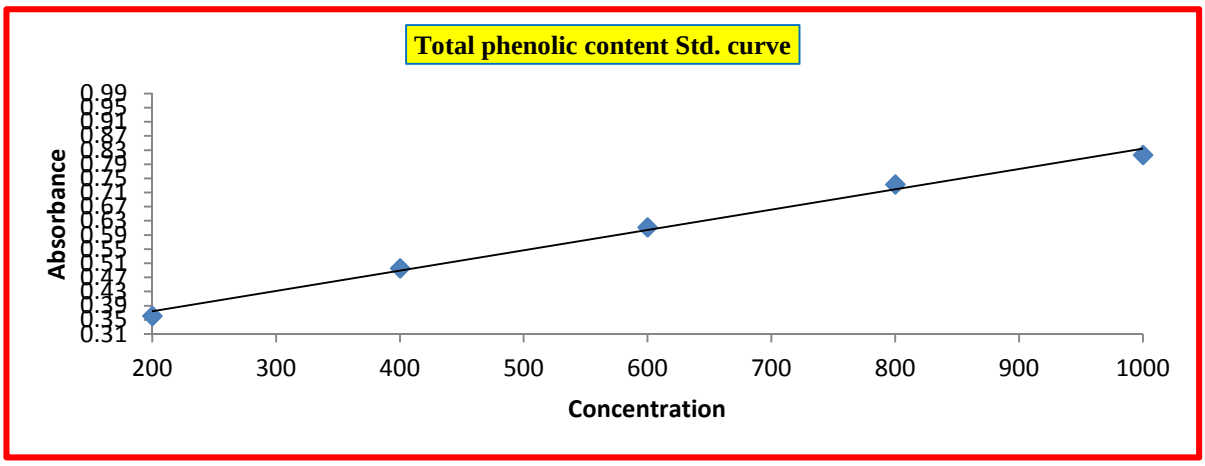
Diabetes mellitus (DM) is a major endocrine disorder characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolisms, which not only lead to hyperglycaemia but also cause many complications such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis[1]. The World Health Organization (WHO) estimates that more than 220 million people worldwide have diabetes and this number is likely to more than double by 2030. The treatment of hyperglycaemia in diabetic patients is directed towards achieving euglycemia and minimizes chronic complications by administering oral hypoglycaemic agents. Despite this, the steady rise of insulin resistance cases of NIDDM is causing grave concern among the physicians. Although a number of pharmacological approaches to the treatment of NIDDM are currently available, it is clear that none is ideal for the treatment of a great majority of NIDDM patients[2]. Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications[3]. In view of the side effects associated with the treatment by insulin and synthetic drugs which are available at present, searching for effective and safer hypoglycaemic plant drugs is going on all over the world[2].

*Adiantum capillus-veneris* Linn. (Adiantaceae) is a medicinal, ornamental, delicate graceful fern, small, rhizomatous, erect and perrenial herb upto 30 cm tall with long polished black stripes widely distributed throughout the world. Ethnomedicinally, the genus is important and popularly known as "Hansraj" in Ayurvedic System of Medicine [4-9]. *Adiantum capillus veneris* Linn. is one of the most common species with potential importance for medicinal and nutritive purpose[10]. Its beneficial effects are observed against dermatitis, diuretic, cystitis, cold, fever, cough, toothache, dental abscesses, gastritis, as stimulant, emollient, purgative, demulcent, general tonic, hair tonic, respiratory problems, tumours of spleen, liver and other viscera[11,12]. It has been used in tea for respiratory diseases and as syrup for severe cough. Also, it promotes hair growth and makes the color of hair black[13]. Concerning the

Table 1: Absorbance of catechin for preparation of standard curve

| Concentration (µg/ml) | Absorbance of Catechin (nm) |
|---|---|
| 200 | 0.362 |
| 400 | 0.496 |
| 600 | 0.612 |
| 800 | 0.734 |
| 1000 | 0.817 |

*Author for correspondence: E-mail: vadiranjan@gmail.com

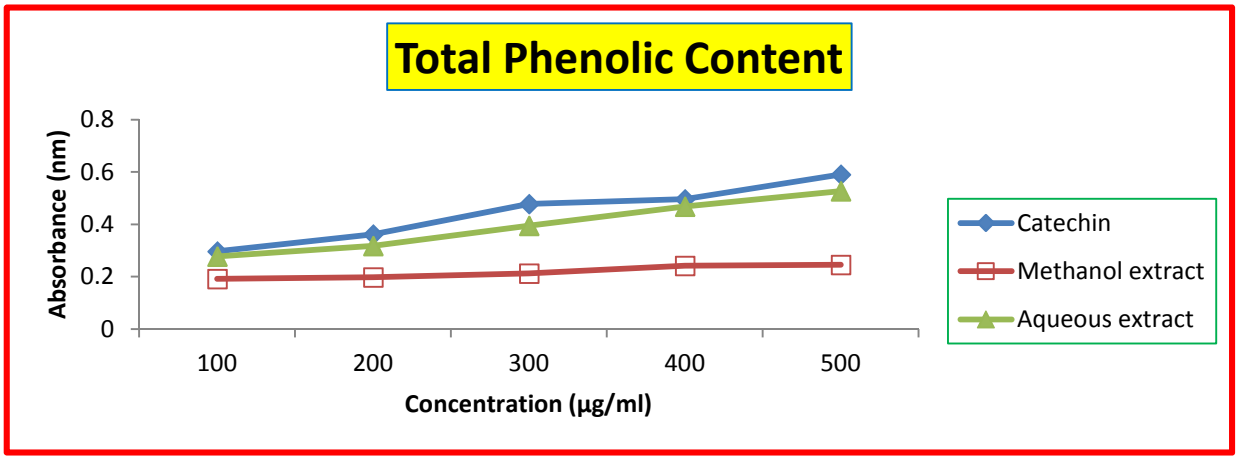



*Graph 1: Catechin standard curve*

Table 2: Observations for absorbance of catechin & *Adiantum capillus veneris* Linn. extracts in total phenolic content determination

| Concentration (µg/ml) | Absorbance (nm) | | |
|---|---|---|---|
| | Catechin | Methanol extract | Aqueous extract |
| 100 | 0.297 | 0.192 | 0.278 |
| 200 | 0.362 | 0.198 | 0.318 |
| 300 | 0.478 | 0.213 | 0.395 |
| 400 | 0.496 | 0.242 | 0.468 |
| 500 | 0.591 | 0.246 | 0.527 |

*# Absorbance is expressed as mean of the absorbances observed in triplicate for each sample & standard.*




*Graph 2: Total phenolic content of Adiantum capillus veneris Linn. extracts & catechin*

Table 3: Total phenolic content of methanol and aqueous extracts

| Concentration (µg/ml) | Methanol ex. (mg. catechin eq./ g dry wt) | Aqueous ex. (mg. catechin eq./ g dry wt) |
|---|---|---|
| 100 | 0.196±0.003*** | 0.281±0.006** |
| 200 | 0.204±0.008*** | 0.321±0.003*** |
| 300 | 0.216±0.003*** | 0.395±0.004*** |
| 400 | 0.246±0.008*** | 0.472±0.005** |
| 500 | 0.254±0.011*** | 0.546±0.020** |

*# Results are expressed as mean ± S.D., *P< 0.05, **P< 0.01, ***P< 0.05 following Dunnett comparison test with standard using one way ANNOVA*

phytoconstituents, the literature revealed the presence of flavonoids, sulphate esters of hydroxycinnamic acid, sugars and different classes of triterpenoids, sterols, bitter material, mucilage, tannins and ester[11, 13]. Because of the high level content of flavonoids and phenols present in it, the biological properties attributed to this species, including anti-inflammatory, anti-infective and anti-tumours may originate from these components and the probable functional mechanism were antimicrobial and antioxidant effects[12]. The present study was aimed to perform a systematic study and to investigate the possible hypoglycaemic and antidiabetic activities of extracts of *Adiantum capillus veneris linn.* in normal and in streptozotocin induced diabetic rats.

Table 4: Observations for absorbance of rutin for preparation of standard curve

| Concentration (µg/ml) | Absorbance of Rutin (nm) |
|---|---|
| 10 | 0.023 |
| 20 | 0.042 |
| 30 | 0.05 |
| 40 | 0.063 |
| 50 | 0.072 |
| 60 | 0.087 |
| 70 | 0.099 |
| 80 | 0.112 |
| 90 | 0.123 |
| 100 | 0.133 |

## MATERIALS AND METHODS

Plant material collection, authentication and preparation of extracts: Disease free dried plant of *Adiantum capillus veneris* Linn. were collected from a commercialized source Verdure Herbals, New Delhi. The plant was authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New-Delhi, under the voucher specimen No.-NISCAIR/RHMD/Consult/2011-12/1792/92 AND and specimen was submitted to the department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonepat, Haryana (India). Successive extracts of *Adiantum capillus veneris* Linn. whole plant powder were prepared using soxhlet apparatus petroleum ether (50-80°C), chloroform and methanol. Aqueous extract was then prepared by boiling the powder with water.

Chemicals and standard drugs: 2-aminoethyl diphenylborinate (Sigma Aldrich), Folin ciocalteau reagent (Central Drug House Pvt. Ltd., New Delhi), rutin and catechin (Yucca Enterprise, Mumbai), sodium carbonate (Merck India Limited, Mumbai), streptozocin (Sigma Aldrich).

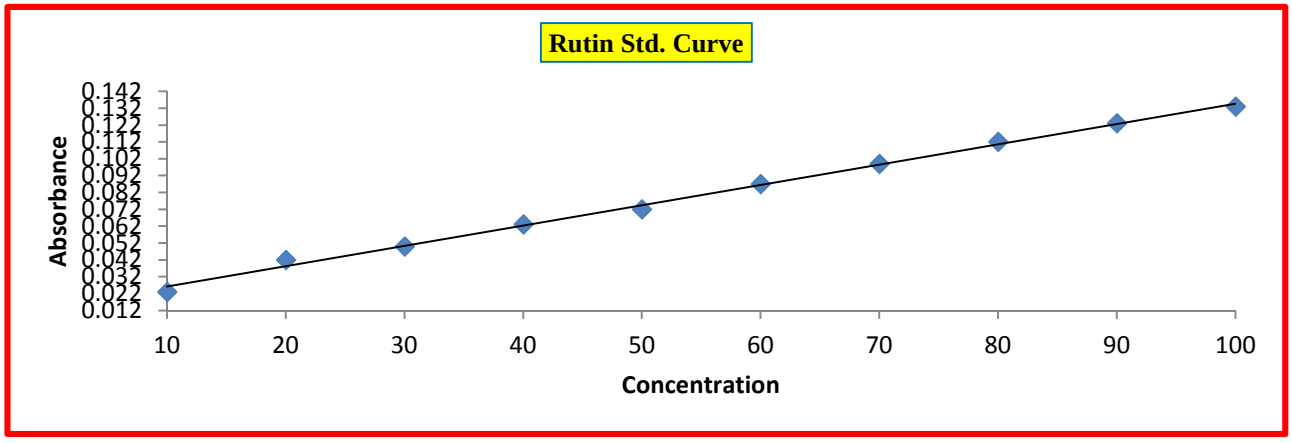
Preliminary Phytochemical Evaluation[14]: Phytochemical screening of various extracts showed the presence of fats, flavanoids, steroids, saponins, tannins and phenolic compounds.

Estimation of Phytoconstituents [15, 16, 17]

Preparation of test Solutions: 1 mg/ml solution of the methanolic and aqueous extracts of *Adiantum capillus veneris* Linn. extracts was used as stock solution. 100, 200, 300, 400 & 500 µg/ml concentrations were used as test solution. Same concentrations were prepared for standard and control also.

Determination of total flavonoid content in plant extracts: The concentration of total flavonoids in the plant extracts was determined by using rutin as reference compound. Standard solutions were prepared by using 10 mg/100 ml solution of rutin as stock solution. 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml concentrations were used for preparation of standard curve. Various samples (test solution of extracts) (0.5 ml) of the methanol and aqueous extracts of whole plant extract of *Adiantum capillus veneris* Linn. were mixed with 4.5 ml water and 0.5 ml of 1% 2-aminoethyl diphenylborinate. The absorbance of the solution was determined at 404 nm using an UV/ visible spectrophotometer.

Determination of total phenolic content in plant extracts: The concentration of total phenols in the plant extracts was determined by using catechin as reference compound. Standard solutions were prepared by using 1 mg/ml solution of catechin as stock solution. 200, 400, 600, 800

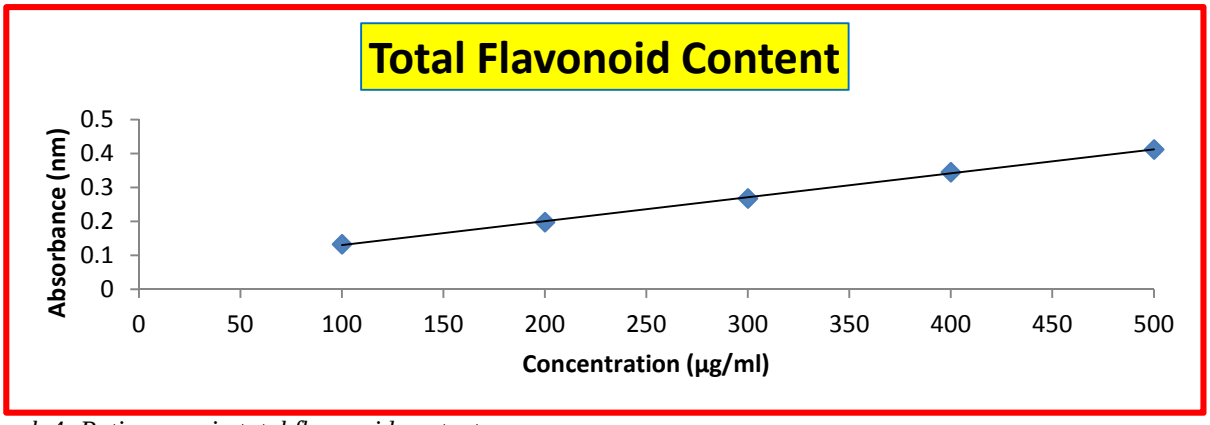



*Graph 3: Rutin standard curve*

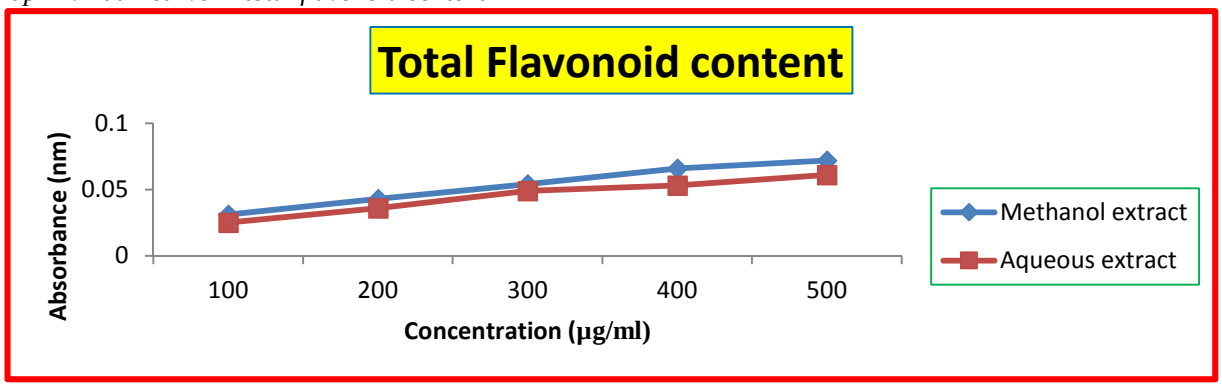
Table 5: Observations for absorbance of rutin & *Adiantum capillus veneris* Linn. extracts in total flavonoid content determination

| Concentration (µg/ml) | Absorbance (nm) | | |
|---|---|---|---|
| | Rutin | Methanol extract | Aqueous extract |
| 100 | 0.133 | 0.031 | 0.025 |
| 200 | 0.198 | 0.043 | 0.036 |
| 300 | 0.268 | 0.054 | 0.049 |
| 400 | 0.345 | 0.066 | 0.053 |
| 500 | 0.412 | 0.072 | 0.061 |

*# Absorbance is expressed as mean of the absorbances observed in triplicate for each sample & standard.*




*Graph 4: Rutin curve in total flavonoid content*




*Graph 5: Total flavonoid content of Adiantum capillus veneris Linn. extracts*

Table 6: Total flavonoid content of methanol and aqueous extracts

| Concentration (µg/ml) | Methanol ex. (mg. rutin eq./ g dry wt) | Aqueous ex. (mg. rutin eq./ g dry wt) |
|---|---|---|
| 100 | 0.0357±0.005*** | 0.0283±0.003*** |
| 200 | 0.0493±0.006*** | 0.0433±0.006*** |
| 300 | 0.058±0.003*** | 0.052±0.003*** |
| 400 | 0.0773±0.01*** | 0.0727±0.023*** |
| 500 | 0.0753±0.003*** | 0.066±0.004*** |

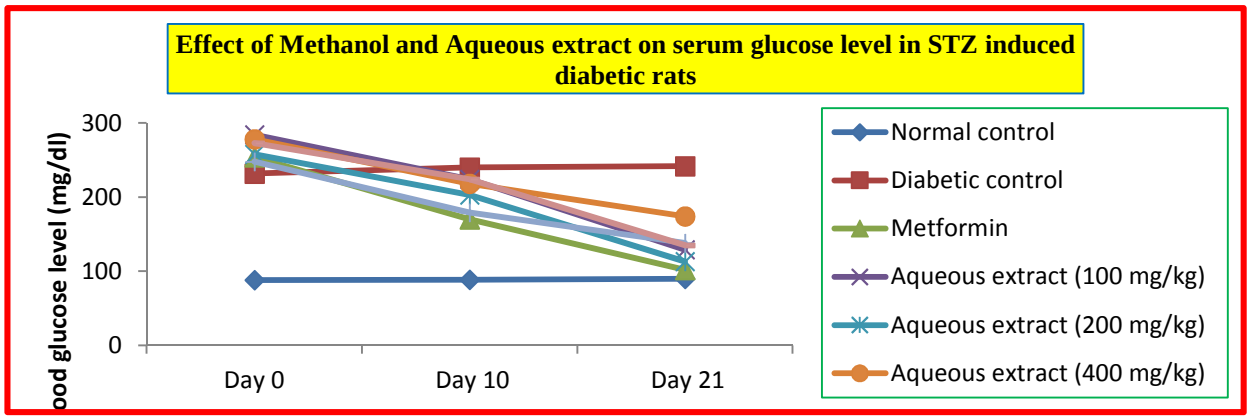
*# Results are expressed as mean ± S.D., *P< 0.05, **P< 0.01, ***P< 0.05 following Dunnett comparison test with standard using one way ANNOVA*

Table 7: Effect of extracts of *Adiantum capillus veneris* Linn. on fasting blood glucose in STZ induced diabetic rats

| Group no. | Treatment | Blood glucose level (mg/dl) | | |
|---|---|---|---|---|
| | | 0th day | 10th day | 21st day |
| 1 | Normal control | 88±2.44 | 88.5±2.42 | 90±2.29 |
| 2 | Diabetic control | 232±24.9** | 240±24.8** | 242±23.4*** |
| 3 | Metformin | 254±23.0** | 170±27.3 | 102±11.1 |
| 4 | Aqueous extract (100 mg/kg) | 284±16.9*** | 224±23.0* | 129±15.0 |
| 5 | Aqueous extract (200 mg/kg) | 258±29.5** | 203±25.0 | 113±7.48 |
| 6 | Aqueous extract (400 mg/kg) | 278±51.7*** | 218±52.1* | 174±49.8 |
| 7 | Methanol extract (200 mg/kg) | 248±28.6** | 179±22.4 | 138±16.2 |
| 8 | Methanol extract (400 mg/kg) | 273±37.9*** | 224±37.4* | 135±21.2 |

*Values are mean ± S.E.M. n= 6 in each group; statistical analysis by one way ANNOVA; significant at p < 0.05 Vs Normal control followed by Dunnet's multiple comparison test; Standard (Metformin) = 50 mg/kg body weight.*

& 1000 µg/ml concentrations were used for preparation of standard curve. Total phenolic content of the methanolic and aqueous extracts of whole plant of *Adiantum capillus veneris* Linn. were measured using Folin ciocalteau reagent. Folin ciocalteau reagent was diluted by 10 times using de-ionized water. The diluted reagent (0.75 ml) was mixed with 0.1 ml sample (test solution of extracts) and




*Graph 6: Effect of extracts of Adiantum capillus veneris Linn. on fasting blood glucose in STZ induced diabetic rats*

held at room temp. for 5 min and then 0.75 ml of 2% sodium carbonate solution was added. After 15 minutes of incubation at room temperature, the absorbance of the solution was determined at 750 nm by UV/ Visible spectrophotometer.

Animals: Healthy albino wistar strain rats of either sex (150-200 gm) were used for the studies. Throughout the experimental period, the animals were housed in colony cages under standard laboratory conditions of temperature (20 to 25 °C), humidity (50-60%) and 12 h light and 12h dark cycle. The animals were provided with food (Golden feed, Delhi) and water *ad libitum*. Approval was taken from the Institutional Animal Ethical Committee (IAEC) of Hindu College of Pharmacy, Sonepat, India under Reg No: 585/02/c/CPCSEA and Item No. – I (3).

Acute Toxicity Studies[18]: Acute oral toxicity was performed as per-OECD 420 guidelines. The animals were fasted overnight before experimentation with free access to drinking water. A total of 10 animals were used divided into two groups consisting of five animals; which will receive a single oral dose (2000 mg/kg body weight) of aqueous and methanolic extracts. Animals were observed individually at least once during first 30 min. After dosing, periodically during first 24 hr (during special attention during first 4 hr) and thereafter once daily for a period of 14 days for major changes in behaviour and mortality.

Hypoglycemic activity[19]: Diabetes was induced intraperitoneally by injecting freshly prepared solution of STZ (45 mg/kg bw) in citrate buffer 0.1 M with ph 4.5 to overnight fasted rats. After 3 days, blood samples were collected from the tail vein, after at least 12 hours of fasted animals. Blood was kept in fluoride tubes. It was centrifuged at 4000 rpm for 10 minutes to obtain clear serum. Glucose levels were estimated using Glucose Oxidase method and body weight was checked regularly up to stable hyperglycemia, after 1 week of STZ injection. The animals having marked hyperglycemia (Fasting blood glucose >250 mg/dl) were selected for the study. Metformin (received as a generous gift from Ranbaxy Labs. Gurgaon, India) was used as the standard drug.

The experiment was carried on eight groups of six rats each and treated orally as follows:

Group I: Normal control: given only vehicle (distilled water)

Group II: Diabetic control: Diabetic given only vehicle (distilled water)

Group III: Diabetic treated with Metformin (50 mg/kg/day)

Group IV: Diabetic treated with aqueous extract (100 mg/kg/day)

Group V: Diabetic treated with aqueous extract (200 mg/kg/day)

Group VI: Diabetic treated with aqueous extract (400 mg/kg/day)

Group VII: Diabetic treated with methanol extract (200 mg/kg/day)

Group VIII: Diabetic treated with methanol extract (400 mg/kg/day)

Blood samples were collected from tail vein at the defined time patterns. Fasting blood glucose levels were estimated on 0, 10$^{th}$ and 21$^{st}$ day of experiment using glucose oxidase method and the readings were observed in autoanalyser. The body weights of all the animals under study were taken gravimetrically.

## STATISTICAL ANALYSIS

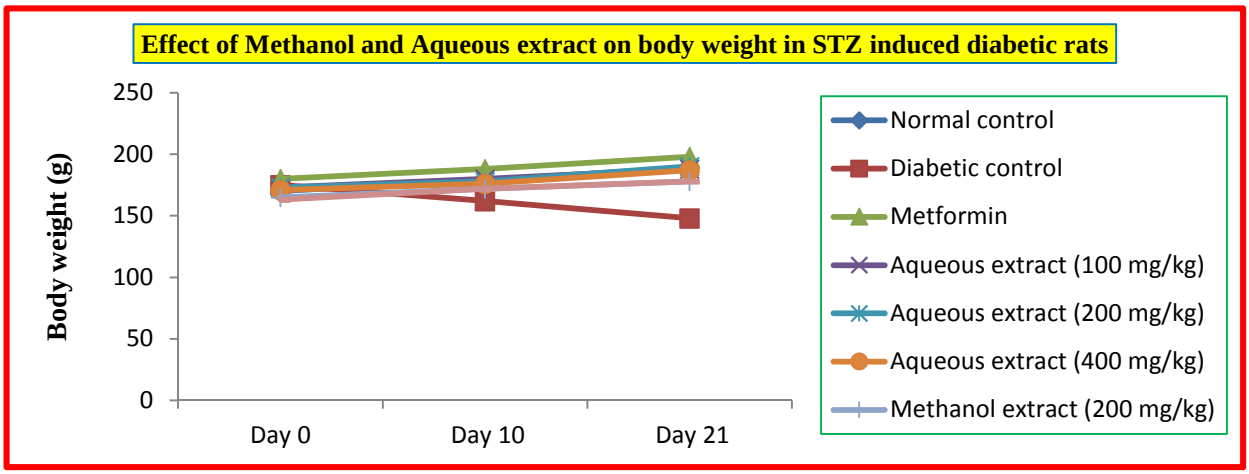
The data are expressed as mean ± standard error mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Dunnet's test. The results having $p < 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

The phytochemical screening of whole plant extracts revealed the presence of phenolic compounds, tannins, flavonoids, carbohydrates, alkaloids, steroids and proteins. Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites universally present in higher plants. While estimating the total phenolic content of *Adiantum capillus veneris* Linn. whole plant extract, aqueous extract showed highest phenolic content at 500 µg/ml followed by methanolic extract (Table 1, 2, 3 and graph 1, 2). Water is good solvent for *Adiantum capillus veneris* Linn. as large amount of phenolics compounds are soluble in water. Phenolic compounds are known as powerful chain breaking antioxidants. Flavonoids are natural phenolic compounds and well known antioxidants. Total flavonoid content was

Table 8: Effect of extracts of *Adiantum capillus veneris* Linn. on average body weight in STZ induced diabetic rats

| Group no. | Treatment | Body weight (g) 0th day | 10th day | 21st day |
|---|---|---|---|---|
| 1 | Normal control | 170±5.16 | 179±4.55 | 189±3.75 |
| 2 | Diabetic control | 175±7.19 | 162±6.01 | 148±7.03*** |
| 3 | Metformin | 180±4.47 | 188±4.22 | 198±4.77 |
| 4 | Aqueous extract (100 mg/kg) | 173±6.67 | 180±5.77 | 188±6.67 |
| 5 | Aqueous extract (200 mg/kg) | 173±6.67 | 178±6.01 | 190±7.75 |
| 6 | Aqueous extract (400 mg/kg) | 171±4.90 | 176±4.36 | 187±6.15 |
| 7 | Methanol extract (200 mg/kg) | 165±4.28 | 172±4.41 | 178±5.58 |
| 8 | Methanol extract (400 mg/kg) | 163±5.58 | 172±4.47 | 178±3.33 |




*Graph 7: Effect of extracts of Adiantum capillus veneris Linn. on average body weight in STZ induced diabetic rats*

determined for methanolic and aqueous extracts of *Adiantum capillus veneris* Linn. at 100-500 µg/ml doses (Table 4, 5, 6 and graph 3,4, 5). The flavonoid content was found to be more in methanol extract than aqueous extract. Acute toxicity studies revealed the non-toxic nature of aqueous and methanol extracts. There were no lethality or toxic reactions found at any doses selected. Antidiabetic activity was carried out for various concentrations of methanol and aqueous extracts of *Adiantum capillus veneris* Linn. in normoglycemic and hyperglycemic rats. The continuous treatment for 21 days with the methanol and aqueous extracts caused a significant decrease in the blood glucose levels of diabetic rats (Table 7 and graph 6). There was gradual increase in body weight in the normal controls while the diabetic control continued to lose weight (Table 8 and graph 7). Treatment with methanol and aqueous extracts decreased the reduction in body weight by diabetes. The capability of *Adiantum capillus veneris* Linn. to protect the body from weight loss seems to be a result of its ability to reduce hyperglycemia. Aqueous extract showed strong antidiabetic effect at low dose of 100 mg/kg b.wt. and methanol extract at high dose of 400 mg/kg b.wt. showed significant antidiabetic effect. From the phytochemical analysis it was found that the major chemical constituents of the extract were flavonoids, steroids and tannins. On the basis of the above evidences it is possible that the presence of flavonoids and tannins are responsible for the observed antidiabetic activity. Further studies are to be carried out to identify the precise site(s), the molecular and cellular mechanism(s) and the active component(s) responsible for the antidiabetic activity.

## CONCLUSION

From this study, we can conclude that methanol extract at high dose of 400 mg/kg b.wt. and aqueous extract at low dose of 100 mg/kg b.wt. has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes.

## REFERENCES

1. Itankar PR, Lokhande SJ, Verma PR, Arora SK, Sahu RA, Patil AT. Antidiabetic potential of unripe *Carissa carandas* Linn. fruit extract. *Journal of Ethnopharmacology* 2011; 135(2): 430-433.
2. Kumar AY, Nandakumar K, Handral M, Talwar S, Dhayabaran D. Hypoglycaemic and anti-diabetic activity of stem bark extracts *Erythrina indica* in normal and alloxan-induced diabetic rats. *Saudi Pharmaceutical Journal* 2011; 19(1): 35-42.
3. Pandit R, Phadke A, Jagtap A. Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* 2010; 128(2): 462-466.
4. Jiang MZ, Yan H, Yan w, Li xm. In vitro and in vivo studies of antioxidant activities of flavonoids from *Adiantum capillus veneris* linn. *African Journal of Pharmacy and Pharmacology* 5(18): 2079-2085.
5. Nakane T, Arai Y, Masuda K, Ishizaki Y, Ageta H, Shiojima K. Fern constituents: six new triterpenoid

alcohols from *Adiantum capillus veneris*. *Chem. Pharm. Bull*. 47(4); 543-547.
6. Chatterjee A, Pakrashi SC. The Treatise on Indian Medicinal Plants,Vol I, CSIR, New Delhi, 2005, 6.
7. Deshpande DJ. A Handbook of Medicinal Herbs, Agrobios, India, 121.
8. Sharma R. Medicinal Plants of India. Daya Publishing House, 2003, 12.
9. Singh M, Singh N, Khare PB, Rawat AKS. Antimicrobial activity of some important *Adiantum* species used traditionally in indigenous systems of medicine. *Journal of Ethnopharmacology* 2008: 327–329.
10. Rajurkar NS, Gaikwad K. Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves. *Journal of chemical and pharmaceutical research* 2012; 4(1): 365-374.
11. Ibraheim ZZ, Ahmed AS, Gouda YG. Phytochemical and biological studies of Adiantum capillus-veneris L. *Saudi pharmaceutical journal* 2011: 65-74.
12. Yuan Q, Wang J, Ruan J. Screening for bioactive compounds from Adiantum capillus veneris L., *J. Chem. Soc. Pak*. 2012; 34(1): 207-216.
13. Besharat M, Rahimian M, Besharat S, Ghaemi E. Antibacterial effects of *Adiantum Capillus Veneris* ethanolic extract on three pathogenic bacteria in vitro. *Journal of Clinical and Diagnostic Research* 2008:1242-1243.
14. Khandelwal KR, Practical Pharmacognosy, ed. 16th, Nirali Prakashan, Pune, 2006, 149-153.
15. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables & grain products. *J Agric Food Chem* 1998; 46(10): 4113-7.
16. Sun T, Powers JR, Tang J. Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food chem*. 2007; 105: 101-106.
17. Oomah BD, Mazza G. Flavonoids and antioxidative activities in buckwheat. *J Agric Food Chem* 1996; 44(7):1746-1750.
18. OECD Guideline for testing of chemicals. Acute oral toxicity- Fixed dose procedure guidelines 420. Dated 17th dec. 2001.
19. A.N. Kesari et. al. *Journal of Ethnopharmacology* 2007; 112: 305-11.