Research Article

# Antidiabetic and Antioxidant Activity of Ethanolic Extract of *Ajuga parviflora* Benth. (Lamiaceae) vern. Neelkanthi, Neelbati

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**ABSTRACT**

The present study was carried out to evaluate the antidiabetic and antioxidant activity of *Ajuga parviflora* (Benth.) ethanolic whole plant extract. *A. parviflora* is a perennial herb with bluish flower belonging to the family Lamiaceae. The *in vitro* analysis of plant extract statistically shows better results as compared to the acarbose which is taken as positive control. *In vitro* studies shows that the % inhibition of alpha-amylase increases as the concentration of the plant extract are increased. *In vivo* effect of plant extract in Wistar male rats shows effective results at 60mg/kg of *A. parviflora* extract and 5mg/kg of glibenclamide given for 14 consecutive days as compared to diabetic control (160mg/kg alloxan). The effect of both the treatments on body weight, blood glucose level, lipid profile, creatinine shows statistically significant results. The antioxidant activity increases as the concentration of the extract are increased. These results indicate that the ethanolic whole plant extract has good antidiabetic and antioxidant potential which can be regarded as a promising candidate for a natural plant source of hypoglycaemic activity and antioxidants. As the antioxidant properties help to neutralize the harmful effects of diabetics on the body.

**Keywords:** *Ajuga parviflora*; alloxan monohydrate; percentage inhibition; antioxidant activity.

## INTRODUCTION

*Ajuga parviflora* (Benth.) is a genus of perennial herbaceous flowering plants in the mint family Lamiaceae native to Europe, Asia, and Africa. In folk medicine, the leaf powder mixed with *Aconitum heterophyllum* (Wall. ex Royle) tuber powder is given a quarter teaspoon twice a day for a long period in the treatment of leucorrhoea, high fever, colic and in diabetes. The plant contains ceryl alcohol, cerotic acid, palmitic acid, oleic acid, linoleic acid, phenolic acids and neutral bitter components, alkaloids, diterpenoids, and triterpenoids.[1] It is observed that in Kullu district of Himachal Pradesh (India) the whole plant of *Ajuga parviflora* has been used for reducing blood glucose level in diabetics.

Diabetes is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.[2] It is considered one of the five leading cause of death in the world.[3] There are two main forms of diabetes. Type I diabetes, that is called Insulin-Dependent Diabetes Mellitus (IDDM) develops when the body's immune system destroys pancreatic β-cells.

The second is characterized by late onset, insensitivity to insulin and partial insulin deficiency. It is generally called Non-Insulin-Dependent Diabetes Mellitus (NIDDM), or type II. Diabetes mellitus is characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia.[4] Autoimmunity, obesity, high carbohydrate diet and sedentary lifestyle are the major causes of diabetes.[5] Postprandial hyperglycemia has been widely reported to be the key feature of impaired glucose tolerance and early diabetes.[6] Therefore a therapeutic approach to treat diabetes is to treat postprandial hyperglycemia.[7] This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha-amylase and alpha-glucosidase. Alpha-amylase and a-glucosidase inhibitors are the major potential targets in the developments of the lead compound of diabetes.

It is accepted that oxidative stress results from an imbalance between the generation of oxygen-derived radicals and the organism's antioxidant potential.[8] People with diabetes tend to have increased generation of reactive oxygen species, decreased antioxidant protection, and therefore increased oxidative damage. The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications. Normal levels of the antioxidants defense mechanism in the body are not sufficient for the eradication of the free radical injury. Therefore the administration of the antioxidant from a natural origin plays a promising role against oxidative stress.

Several antioxidants of plant material have been experimentally proven and widely used as a more effective agent against oxidative stress.[9] It is generally assumed that frequent consumption of plant-derived phytochemicals like phenolics and flavonoids from vegetables, fruit, tea and herb have good antioxidant status.[10]

In this investigation, we have chosen a frequently prescribed plant for the treatment of diabetes by the local healers, elderly people, and the vaids of the study area. We studied the *In vitro* ability of the *A. parviflora* to inhibit the activity of pancreatic alpha-amylase and also

the *In-vivo* antidiabetic activity on alloxan induced diabetic rats. The antioxidant effect of the ethanolic extract of *A. parviflora* has been conducted.

## MATERIALS AND METHODS

### Collection and Identification

The whole plant material was collected from Kullu district, Himachal Pradesh (India) at an altitude of 2200 meters and was identified through diagnostic keys, Flora and authenticated from the Herbarium of Punjab University Chandigarh (PAN) and also deposited in PAN (21076) Chandigarh.

### Preparation of Plant Extract

The whole plants of *A. parviflora* were washed thoroughly with tap water and then with distilled water and shade dried for 3 to 4 days. They were then dried in the oven and ground in a grinder to make fine powder. 25g of powdered material was extracted with 50ml of 90% of ethanol in the shaker for 24 hr. The extract was filtered through Whatman filter paper and the filtrate was evaporated at room temperature. The residue remaining was used as plant extract and was stored at 4 °C for further use.

### Estimation of Antidiabetic Activity

#### *In vitro antidiabetic activity by alpha-amylase inhibition assay*

The alpha-amylase inhibition assay was adopted and modified from Sigma-Aldrich.[11] The 25ml of 1% (w/v) starch solution was prepared in phosphate buffer (pH 6.9). Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating plate using constant stirring for 15 minutes. The starch solution was cooled to room temperature and after cooling, the solution was brought to its original volume. The enzyme solution was prepared by mixing 0.001 g of alpha-amylase (EC 3.2.1.1) in 100 mL of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 mL), 5.31 M sodium potassium tartrate in 2M sodium hydroxide (8 mL) and deionized water (12 mL). The color reagent was diluted to 40 ml.

1mL of the extracts of various concentration (50-250µg) were prepared in DMSO. The absorbance value was determined at 540nm. Acarbose solution was used as positive control. Milligrams of maltose liberated was calculated by using the standard curve of maltose and alpha-amylase inhibition % was calculated according to the formula:

$$Inhibition\ \% = \frac{Abs_{540}(control) - Abs_{540}(test)}{Abs_{540}} \times 100$$

#### *In vivo antidiabetic activity in alloxan-induced rats*

### Animals

Wistar male rats, 8-10 weeks were obtained from Central Animal House Panjab University Chandigarh (45/99/CPCSEA). Rats were fed with standard rat diet (Ashirwad Industries, Tirpari – Kharar District Mohali (Punjab) and water ad libtum. Before initiation of the experiment, the rat was acclimatized for a period of 7 days under standard environmental conditions of temperature i.e 25°C and 12 hrs of light and dark cycle. All protocols for their use in this investigation were approved by Institutional Animal Ethics Committee (IAEC), Central Animal House, Panjab University, Chandigarh.

### Experimental design

Four groups of rats, five in each received the following treatment schedule:

Group I: Normal control

Group II: Alloxan treated control (160 mg/kg.ip)

Group III: Alloxan (160 mg/kg.ip) + *A. parviflora* whole plants extract (60 mg/kg, p.o)

Group IV: Alloxan (160 mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o)

Whole plant extract and standard drug glibenclamide (5 mg/kg) were administered with the help of feeding cannula. Group I, serve as normal control which receives normal water for 14 days. Group II as diabetic control, Group III and Group IV were given a fixed dose of plant extract and standard drug according to their body weight respectively for 14 consecutive days.

### Induction of Diabetes in Experimental Animals

Before induction of diabetes, the rats were kept on fasting for 16 hrs. Animals were made diabetic with a single intraperitoneal injection of 160 mg alloxan/kg body weight in 154Mm saline. They had free access to food and water after 3hrs of intraperitoneal injection. The animals were allowed to drink 5% glucose solution overnight to overcome the hypoglycaemic shock.

The rats were identified as diabetic on the basis of blood glucose levels (ranging from 180 to 300 mg/dl and above at least 2 days post-alloxan treatment). The body weight and blood glucose level of each rat were determined on the 1$^{st}$, 7$^{th}$ and 14$^{th}$ day. Blood glucose level was determined by Code free Glucometer.

The body weight and glycaemic change were calculated according to the formula:

$$Change\ in\ body\ weight\ (bw)\% = \frac{(body\ weight\ on\ 14th\ day - initial\ body\ weight)}{Initial\ body\ weight} \times 100$$

$$\%\ glycaemic\ change = \frac{(glucose\ level\ on\ 14th\ day - Fasting\ blood\ glucose\ level\ on\ the\ first\ day)}{Fasting\ blood\ glucose\ level\ on\ Initial\ day} \times 100$$

For biochemical analyses, blood samples were collected from retro-orbital plexus under mild ether anesthesia. It was allowed to coagulate at room temperature and centrifuged at 3000 rpm for 30 min.

Serum was separated and analyzed for serum cholesterol[12], serum triglycerides by enzymatic DHBS colorimetric method[13] HDL[14], LDL[15], VLDL[15] serum creatinine.[16]

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique.

Sections of 5µm thickness were cut and stained with hematoxylin and eosin (H & E) and histopathological observations were made.

### *In vitro* Antioxidant Activity

The free radical scavenging capacity of ethanolic extract of *A. parviflora* was determined by using DPPH.[17] 0.1mM DPPH solution was prepared freshly in methanol.

The different volume of plant extracts was made (50µg/ml to 250µg/ml) with DMSO and 2.7ml of DPPH (0.1mM) solution was added. After 30 minutes, the absorbance of the mixture was read at 517 nm. Ascorbic acid was used as a standard. DPPH solution was taken as control. Methanol was served as blank. The percentage inhibition is calculated as following formula:

$$\% \, inhibition = \frac{control - test}{control} \times 100$$

### Scavenging of Hydrogen Peroxide

The ability of the extract to scavenge hydrogen peroxide was determined by the standard method.[18-19]

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The plant extract (50-250µg/ml) in methanol were added to a hydrogen peroxide solution (0.6ml, 40mM). The absorbance at 230 nm was determined after 10 minutes against a blank solution contain. Ascorbic acid was taken as standard.

The % of hydrogen peroxide scavenging by the extract and standard compound was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = \frac{A_0 - A_1}{A_0} \times 100$$

Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance of the test.

### Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed by ANOVA followed by post hoc Dunnett t-test. Differences between groups were considered significant at $P<0.05$ levels.

## RESULTS

### Alpha-amylase Inhibition Activity

In this study, the ethanol extract of *A. parviflora* belonging to Lamiaceae was evaluated for their possible alpha-amylase inhibitory activity alongside acarbose as a positive control, the effect on alloxan induced diabetic rats and for their antioxidant activity. Alpha-amylase is one of the main enzymes in human that is responsible for the breakdown of the starch to more simple sugar. Thus the inhibitor of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Also, they can reduce the postprandial plasma glucose level and improve the glucose tolerance in diabetic patients.[20] The alpha-amylase inhibitory activity and $IC_{50}$ values of *A. parviflora* and acarbose were summarized. In acarbose, it was 184.54±6.43 µg/ml (Table 1) and 110±5.02 µg/ml for ethanol extract of *A. parviflora*. The ability of ethanolic plant extract of *A. parviflora* to inhibit the alpha amylase was calculated as percentage inhibition which was found 79.29% at 250 µg/ml, where as the % inhibition for acarbose at the same concentration was 62.60% (Figure 1).

### Effect of EtPE on Body Weight in Experimental Groups

There is a slight increase in body weight in normal control and this may be due to normal growth.

The diabetic control reduced the body weight by 19.32%. In group 3 and 4, the body weight increased by 0.48% and 1% respectively (Table 2).

### Effect of EtPE on Blood Glucose Level

The ethanolic plant extract of *A. parviflora* had a significant hypoglycaemic effect. The blood glucose level was decreased and observed by 59.56% on the 14th day at 60mg/kg concentration. The blood glucose level on the 14th day was 129±1.36 (normal control), 501±3.54 (diabetic control), 185±4.60 (diabetic rats treated with ethanolic plant extract), 185±3.23 (diabetic rats treated with glibenclamide) Table 2.

**Table 1:** Percentage of alpha-amylase inhibition and $IC_{50}$ values by ethanolic extract of *Ajuga parviflora* at various concentration.

| Concentration (µg/ml) | % Inhibition by *A. parviflora* | $IC_{50}$ (µg/ml) *(A. parviflora)* | % Inhibition by Acarbose | $IC_{50}$ (µg/ml) (Acarbose) |
|---|---|---|---|---|
| 50 | 21.23 | 110.18 ± 5.02 | 13.35 | 184.54 ± 6.43 |
| 100 | 45.38 | | 17.12 | |
| 150 | 63.37 | | 40.84 | |
| 200 | 73.32 | | 55.73 | |
| 250 | 79.29 | | 62.60 | |

**Table 2:** Effect of Ethanolic Extract of *A. parviflora* on average body weight and blood glucose level of alloxan induced Diabetic Wistar rats.

| Group | Average body weight (gm) and blood glucose level(mg/dl) | | | | | | Change in % | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | | Day 7 | | Day 14 | | bw % | gc % |
| | gm | mg/dl | gm | mg/dl | gm | mg/dl | | |
| Normal control | 252±7.35 | 128±2.27 | 259±6.78 | 127±2.09 | 265±3.87 | 129±1.36 | 5.16% | 0.78% |
| Diabetic control (alloxan 160mg/kg) | 210±5.40[a] | 424±10.77[d] | 188±4.90[c] | 470±6.94[cd] | 176±5.10[cd] | 501±3.54 | -19.32% | 18.16% |
| Alloxan (160mg/kg)+EtPE (60mg/kg) | 209±6.70[a] | 455±13.92 | 198±5.10[a] | 336±13.27[b] | 210±4.47[b] | 184.60±4.60[a] | 1% | -59.56% |
| Alloxan (160mg/kg)+glibenclamide (5mg/kg) | 200±3.10[a] | 472±7.54[b] | 192±3.74[a] | 362±13.91[b] | 201±7.86[b] | 185±3.23[a] | 0.48% | -60.80% |

*bw-body weight; EtPE-ethanolic plant extract; gc glycaemic change; Each value represents as mean±SEM of 5 observations (ANOVA followed by Dunnett's T test) where values are statistically significant at P<0.05.; [a] P<0.05 when compared with normal control; [b] P<0.05 when compared with diabetic control; [c] P<0.05 when compared with alloxan-induced diabetic rats + EtPE**; [d] P<0.05 when compared with alloxan-induced diabetic rats + glibenclamide

**Table 3:** Effect of ethanolic extract of *A. parviflora* on lipid profile and creatinine in alloxan induced diabetic Wistar rats after 14 days of treatment

| Group | TC (mg/dl) | TG (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | Creatinine (mg/dl) |
|---|---|---|---|---|---|---|
| Normal control | 60.43 ± 4.14 | 80 ± 5.77 | 66.33 ± 0.88 | 90.09 ± 0.57 | 22.43 ± 0.97 | 0.56 ± 0.07 |
| Diabetic control | 79 ± 1.91[c,d] | 169 ± 6.08 | 44.33 ± 1.86[a,c,d] | 126.12 ± 0.31[c,d] | 30 ± 1.15[a] | 2.20 ± 0.12[a] |
| Alloxan + PE | 62 ± 1.58[b] | 110 ± 5.77 | 51 ± 1.85[b] | 100.90 ± 0.33[b] | 24.33 ± 1.45[a] | 0.61 ± 0.10[b] |
| Alloxan + glibenclamide | 66.33 ± 1.6[b] | 107 ± 6.43 | 53 ± 2.88[b] | 113.51 ± 0.33[b] | 25.67 ± 0.88 | 0.56 ± 0.36[b] |

TC-Total cholesterol, TG- Triglycerides, HDL-High Density Lipoprotein, LDL- Low-Density Lipoprotein, VLDL-Very Low-Density Lipoprotein, PE-Plant extract; Each value represents the mean ± SEM of 5 observations (ANOVA followed by Dunnett's T test).

[a] P<0.05 when compared with normal control; [b] P<0.05 when compared with diabetic control; [c] P<0.05 when compared with alloxan-induced rats +PE; [d] P<0.05 when compared with alloxan-induced rats+ glibenclamide

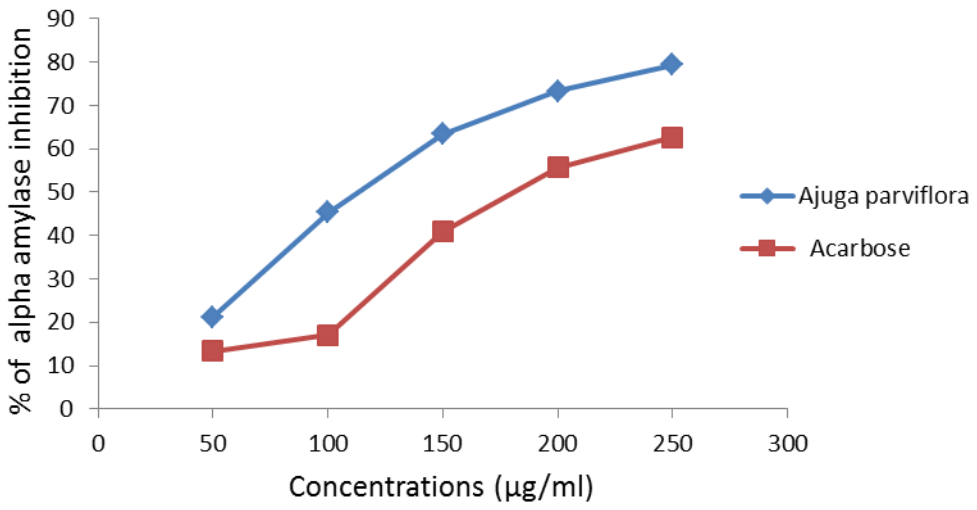
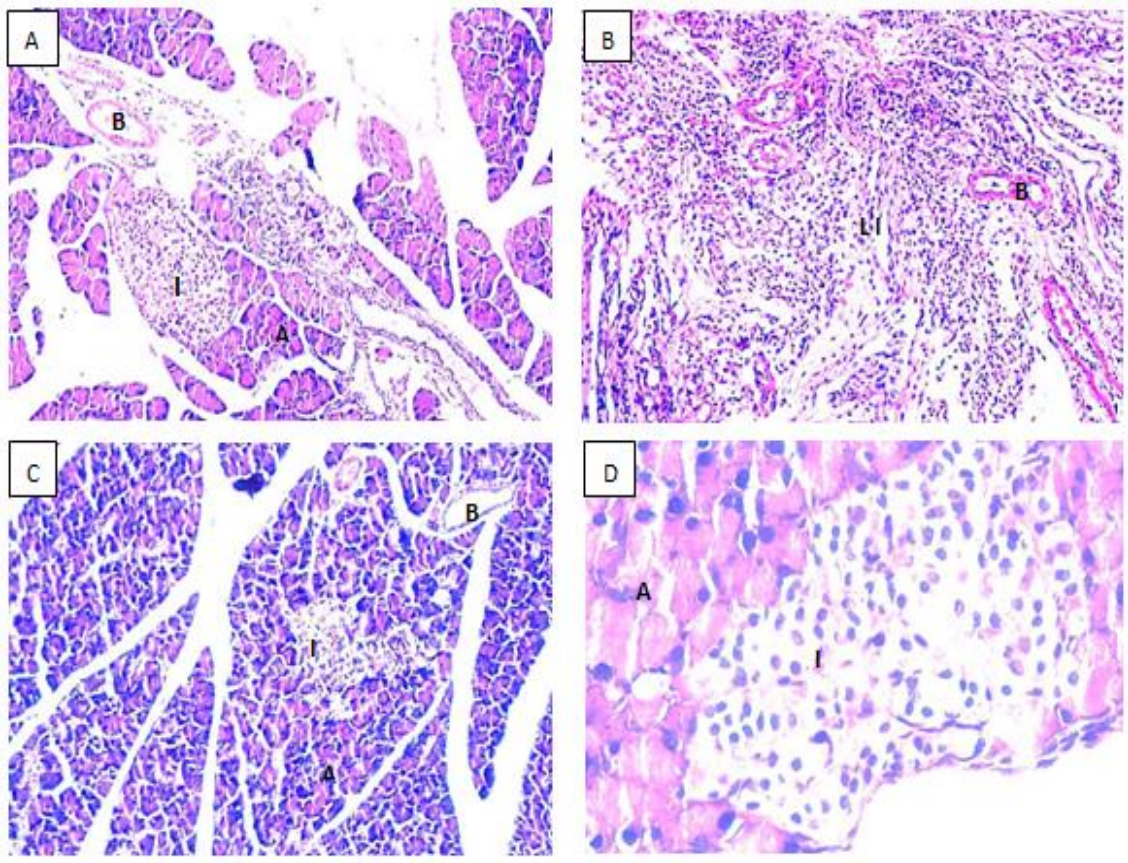



**Figure 1:** % inhibition of the alpha-amylase enzyme by ethanolic extract of *A. parviflora* and reference alpha-amylase inhibitor, Acarbose (values represent Mean ± SEM of three replicates).




**A**- Acinar cell, **B** – Blood vessel **I**- Islets of Langerhans, **LI -** Lymphocyte Infiltration

**Figure 2:** Histopathological studies of rat pancreas stained with hematoxylin –eosin stain A) Normal control with 2-3 islets of Langerhans, acinar cell and blood vessel, B) Diabetic control with inflammatory area, rupture of islets and leucocyte infiltration, C) Diabetic rats treated with ethanol extract of *A. parviflora* shows recovered blood vessel and less infiltration of leucocyte, D) Diabetic rats treated with reference drug (glibenclamide) shows improved islets with a large proportion of a smaller volume as compared to control. There is very scanty inflammatory cell infiltration.

### Effect on Lipid Profile and Creatinine

Alloxan treatment will increase the serum cholesterol, serum triglycerides, LDL and reduction in HDL, but plant dose and glibenclamide reversed the above alloxan induce changes (Table 3).

### Histopathological Observations

Histopathological studies (Figure 2) showed normal acini cell and normal distribution of islets of Langerhans in normal control. Abnormalities such as fewer islets of Langerhans, lymphocyte infiltration were observed in diabetic control. In plant dose and glibenclamide treated group the acinar cells are seen to be normal, islets are present.

### DPPH Free Radical Scavenging Activity

The ability of ethanolic plant extract of *A. parviflora* to scavenge DPPH free radical was calculated by percentage inhibition which was found to be 97.20% at concentration 250µg/ml, whereas at same concentration it was found 92.87% for ascorbic acid (Figure 3).

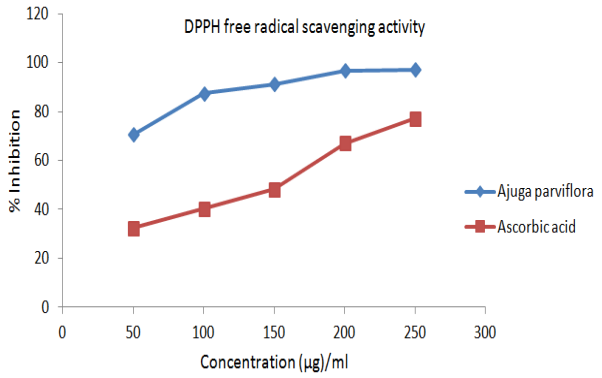



**Figure 3:** % Inhibition of DPPH by ethanolic extract of *A. parviflora* and positive control acarbose (values represent mean ± SEM of three replicates).

### Scavenging of Hydrogen Peroxide

The scavenging ability of ethanol extract was calculated as percentage inhibition. At 250µg/ml it was found to be 92.33% for *A. parviflora* and 73.57% for ascorbic acid (Figure 4).

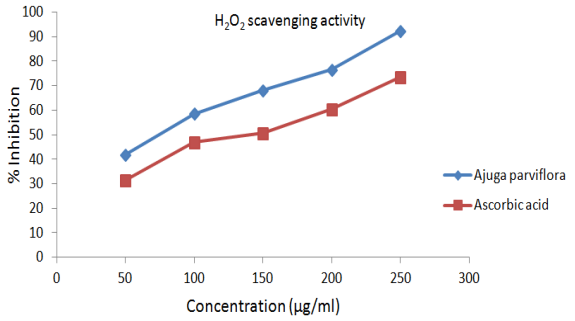



**Figure 4:** Scavenging % of $H_2O_2$ by ethanolic extract of *A. parviflora* and positive control Acarbose (values represents mean ± SEM of three replicates).

## DISCUSSION

The results of our study indicate that the ethanolic extract of *A. parviflora* has good antidiabetic activity. There are no previous reports of alpha-amylase inhibitory activity and antioxidant activity of *A. parviflora*.

Therefore the present experimental study was conducted to find out the possible antihyperglycaemic mechanism of the ethanolic extract of *A. parviflora*.

One of the important goals in the treatment of diabetes is to maintain fasting and postprandial blood glucose near normal levels.[21] The enzyme a-amylase is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose prior to absorption. Therefore presence of such inhibitors in plant extracts may be responsible for impaired starch digestion and the slow down the postprandial blood glucose level.[22] The % inhibition of alpha-amylase by *A. parviflora* was found 79.29% at 250µg/ml, which was statistically significant as compared to Acarbose at the same concentration (Figure 1).

After treatment with the EtPE of *A. parviflora* in alloxan induced diabetic rats, the body weight, blood glucose level and serum lipid profile and histological characters shows normal level. In diabetic rats, the body weight decreased as diabetes progresses.[23] The control groups, plant dose treated as well as reference drugs treated groups shows the increase in body weight and lower level of blood glucose after the treatment and the increase in body weight and a decrease in blood glucose level after the treatment was statistically significant (Table 2). There is an increase in total cholesterol level, triglycerides, LDL, VLDL,creatinine and decrease in HDL in alloxan induced diabetic rats. The lipid profile and creatinine level come to normal in EtPE of *A. parviflora* and glibenclamide treated groups. The increase and decrease in the biochemical analyses are found to be statistically significant when compared to the control. The renewal of beta cells has been studied in several animal models. In our study, the damage of pancreas in alloxan induced diabetic control rats (Figure 2, group 2) and regeneration of beta cells by glibenclamide (Figure 2, group 4) and EtPE of *A. parviflora* (Figure 2, group 3) was observed. The (60mg/kg) plant dose shows the similar effect as that of glibenclamide. This could be due to the possibility that some beta cells are still surviving to act upon by *A. parviflora* extract to exerts its insulin-releasing effect. Similar effects in alloxan-treated plants were reported by *Vinca rosea* extract.[24]

DPPH is one of the powerful free radical which is used to evaluate the electron donating capacity of antioxidants.[25] DPPH is very stable free radical and freshly prepared DPPH solution has a deep purple color with an absorption spectrum of 517 nm. The antioxidants compounds lead to the fadedness of purple color to yellow or colorless, which leads to the decrease in absorbance and hence provides antioxidant potential. The ability of EtPE of *A. parviflora* to scavenge DPPH free radical increases with increase in the concentration of plant extract which was found 97.20% at 250µg/ml, whereas for ascorbic acid it was found 92.87% at the same concentration (Figure 3) and was found statistically significant.

The hydrogen peroxide scavenging ability of EtPE of *A. parviflora* and ascorbic acid is shown in (Figure 4). $H_2O_2$ is a weak oxidizing agent that inhibit the oxidation of essential thiol (-SH) groups directly by few enzymes. It has the ability to cross the cell membrane and once inside the cell, it can probably react with $Fe^{+2}$ and possibly $Cu^{+2}$ ions to form hydroxyl radicals.[26] The $H_2O_2$ scavenging ability of EtPE of *A. parviflora* was found to be 92.33% and 73.57% for ascorbic acid at 250µg/ml. These results suggest that ethanolic extract of *A. parviflora* whole plant can be a better antioxidant for removing $H_2O_2$ and thus protects the living system from oxidative stress.

## CONCLUSION

The whole plant extract shows the good inhibitory effect on alpha-amylase and has effective antioxidant activity. It also lowers the blood glucose level in diabetic rats after 14 consecutive days of treatment.

There is an increase in serum cholesterol, triglycerides, LDL, VLDL and the level of HDL and creatinine decreases in the diabetic rats treated with the plant dose and standard drug and all the results were statistically significant. These results show that the ethanolic whole plant extract of *A. parviflora* can be used for the treatment of diabetes Type II.

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