In-vitro Antioxidant Activity and Antidiabetic Effect of Ethanol Extract of Achyranthes aspera on Wistar Albino Rats.

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Abstract

Herbal medicine is widely used since the immemorial indicating that herbs are growing part of modern, high-tech medicine. The medicinal plants are used for treatment of various diseases because of their safety and effectiveness. The present study, aims to determine the antioxidant activity of Achyranthes aspera extract and to evaluate the antidiabetic effect of aqueous extract of Achyranthes aspera Linn. against alloxan induced diabetic rats. Albino rats were divided into six groups comprising of two rats each. Group 1 to 4 received aqueous extract of A. aspera Linn. against a dosage of 500mg/kg body weight to 62.25mg/kg body weight for 10days respectively. Group 5 received standard drug as control (diabinase 1mg/kg body weight) and group 6 served as negative control which received normal saline (1mg/kg body weight). Alloxan induced diabetic and normal rats were administered orally with aqueous and ethanol extract of A. aspera at different concentration.

At the end of 10days treatment, blood glucose level and body weight were estimated and compared to normal and diabetic control groups. The results show a dose-dependent significant 112.5 ± 3.54, (P<0.05) reduction in blood glucose as well as a significant increase in body weight of treated diabetic rats compared with the diabetic control groups which was comparable to the diabinase-treated group. This study revealed significant hypoglycemic effects of A. aspera, hence justifies the use of A. aspera in ethno-medicine for treatment of diabetes. Also, A. aspera extract exhibited significant antioxidant activity and presence of high level of phenolic compounds. In conclusion A. aspera leaves possess high antioxidant activity and can be explored as a source of natural antioxidant compounds.

Keywords: Medicinal, Antioxidant, Diabetic, Hypoglycemic, Herbs, Achyranthes aspera, Albino Rats.
Introduction

Oxidation is a normal physiological and metabolic process in the cell. During the process, approximately 5% oxygen gets reduced to the oxygen based free radicals, includes superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals (Gutteridge and Halliwell 2004). These free radicals are collectively known as Reactive Oxygen Species (ROS). Free radicals formed during metabolism are electrically charged and react with nucleic acids, mitochondria, proteins and enzymes and resulting in their damage (Halliwell and Gutteridge, 2009, Morrissey and O’Brien 2008). The antioxidants comprise of a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules, such as proteins, lipids, CHO and DNA. Many of these substances which contribute to the prevention of treatment of diseases, in which reactive species of oxygen are involved, are natural molecules of plants. This protection can be explained by the capacity of the antioxidants of plants to scavenge free radicals. The antioxidant defense mechanism is affected by age, diet and health condition of individual (Yu B.P. 2004). When formation of free radicals start attacking the cell and resulting in several physiological disorders like Alzheimer’s disease, cancer, atherosclerosis, diabetes, liver cirrhosis and rheumatism (Frankel et al. (2003); Godwin and Brodwick, (2005), Hatt, K. and Kim, H. (2009).

The plant polyphenols may act as potent antioxidant and can prove beneficial for maintenance of optimal health, further assistance in providing evidence for the claimed medicinal properties of Achyranthes aspera. One of the most dangerous and life threatening diseases in the world today, involving the pancreas, is Diabetes mellitus (Verbrugge et al. 2009). Diabetes is a complex disease which is characterized by grossly abnormal fuel usage: glucose is overproduced by the liver and underutilized by other organs. The incidence of diabetes mellitus (usually referred to simply as diabetes) in industrialized countries is about 1%. Indeed, diabetes is the most common serious metabolic disease in the world; it affects hundreds of millions. Type 1 diabetes is caused by auto-immune destruction of insulin-secreting β-cells in the pancreas. Type 2 diabetes, by contrast, has a different cause. A genetic basis seems likely but the molecular lesion has not been identified (Lubert Strayer 2005). Diabetes is a condition primarily defined by the level of hyperglycemia giving rise to risk of is the micro vascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related micro vascular complications increased risk of macro vascular complications. (Ischemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. Diabetes is a syndrome characterized by disordered metabolism of carbohydrates, protein and lipid with abnormally high blood sugar (hyperglycemia) resulting from low levels of hormone insulin with or without abnormal resistance to insulin’s effect. The record of its prevalence in the world today and the increase in morbidity and mortality rates calls for an urgent attention from the health professional’s policy makers and executioners in tackling this disease.

In Cote d’Ivorie, the rate of morbidity is 5.7% (Zinirou, 2007). Traditional medical herbs used for strengthening the body immune system and for therapeutic purposes are known to have many essential and nutritional elements. Many plants have been documented to have the efficacy to treat diabetes all over the world (Akerele, et al., 2011). The currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin are considered to have limitations of their own, hence herbal medicines have been recommended for treatment of diabetes. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been empirically as anti-diabetic and antihyperlipidic remedies. Antihyperglycemic effect of these are attributed to their ability to restore the function of pancreatic tissues by causing increase in insulin output or by inhibiting the intestinal absorption of glucose or by facilitating the metabolites in insulin dependent processes.

Materials and Methods

Fresh leaves of Achyranthes aspera were collected from places in and around Amaoba and at National Root Crops Research Institute, Umudike, Nigeria. The sample of the plant was identified and authenticated by a Botanist Dr. Osuagwu from Michael Okpara University of Agriculture, Umudike. The leaves were sorted to eliminate any dead and unwanted particles. The leaves were washed thoroughly and rinsed severally with tap water to remove dust particles and debris and thereafter allowed to sun-dried for two weeks. The leaves were blended with a hand blender and 100g of the plant was weighed and soaked in 500ml of ethanol. Homogenate were stored in amber colored bottle in the dark at 4°C for 72hours. These were then filtered using a cheese material and thereafter with Whatman No 1 filter paper to obtain a homogenous filtrate. The filtrate was then concentrated in vacuo at low temperature of 37 to 40°C. The concentrate was then allowed in water bath for complete dryness of greenish brown and brown oily substance of Achyranthes aspera the extract was refrigerated at 2 - 5°C until when used.

Twelve adult Wister’s rats (150-200g) of female sex obtained from the animal house of Department of Biochemistry, University of Nsuka, Nigeria, were used for the experiment. The rats were acclimatized in the experimental animal house for two weeks before the commencement of the experiment. The animals were housed in stainless steel cages under standard conditions. (Ambient temperature 28.0 ± 2.0°C and humidity 46% with a 12 h light/dark cycle), were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water ad libitum, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. Prior to diabetes induction, the rats were fasted overnight, and then diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan (154 mg/kg body weight) in ice cold 0.9% sodium chloride (NaCl) saline solution. The rats were considered as being diabetic if blood glucose values, as estimated using One Touch Glucometer (Lifescan, California, USA) on blood obtained from the tail vein of the overnight fasted rats, were above the range of 200mg/dl on the third day after alloxan injection. The leaf extract of the plant in aqueous solution were administered to rats orally through a gavage at a concentration of 100/kg body weight/rats/day for ten days.
Experimental Design

Twelve rats were divided into six groups of two rats each consisting of diabetic (DC) and non-diabetic (NC) control group. Before use, the extract were reconstituted in distilled water and administered orally via gavage, at dose of 500mgkg⁻¹ and 250mgkg⁻¹, 125mgkg⁻¹, 62.5mgkg⁻¹ respectively. The positive control received diabinase while negative control received distilled water. The animals were maintained on pelleted Growers Feed obtained from Vital Feeds, Umuahia, Nigeria, and tap water. Both the feed and water were provided ad libitum and treatment lasted for ten days.

The rats were divided into six groups of two rats each and treated as follows:

Group I: Diabetic rats administered orally with leaf extract at dose of 500 mg/kg/day in aqueous solution for ten days.

Group II: Diabetic rats given leaf extract at a dose of 250 mg/kg/day in aqueous solution orally for ten days.

Group III: Diabetic rats administered orally with leaf extracts at a dose of 125 mg/kg/day in aqueous solution for ten days.

Group IV: Diabetic rats administered orally with leaf extract at a dose of 62.5 mg/kg/day in aqueous solution for ten days.

Group V: Diabetic rats given diabinase for ten days in aqueous solution orally for ten days as positive control.

Group VI: Diabetic rats given water for ten days as negative control.

The fasting BGLs (blood glucose level) of all the rats were recorded at regular intervals during the experimental period. For acute study, the BGL was monitored every day of administration of the dose of the extract and at the end of ten days for prolonged treatments. The BGL was monitored in the blood of the diabetic rats by tail tipping method. The blood was dropped on glucometer stripes which were inserted into the glucometer and the readings were noted and recorded.

Evaluation of radical scavenging activities by antioxidants in the plant extract was carried out using DPPH (1, 1-diphenyl-2-picrylhydrazly) radicals (Brand- William et al 1995). Different volumes of 0.2 mg/ml of the plant extract were added to 200µl of (0.36 mg/ml concentration) DPPH solution in methanol. A series of concentration ranging from 2 to 15µg of dried extract were tested. The mixtures were vigorously shaken and incubated in the dark for 30minutes after which the reduction of DPPH absorption was measured at 517nm. Percent inhibition by sample treatment was determined by comparing it with the methanol- treated control group. The ability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging activity (%) = (A₀ - A₁)/ (A₀ × 100) where A₀ is the absorbance of the control and A₁ is the absorbance of the sample. The IC₅₀ values denote the concentration of each sample required to give 50% of the optical density shown by the control, using a non-linear regression analysis. All test analysis were run in duplicates and both values were reported. Ascorbic acid (300 mg/ml) was used as the positive control.

The nitric oxide scavenging activity was conducted based on the Greiss illosvosy reaction, (Green et al 1982). 2.0mL of 10Mm sodium nitroprusside and 5.0mL of phosphate buffer were mixed with 0.5mL of different concentrations of the seed extract and incubated at 25°C for 150 minutes. The sample was run and the blank was replaced with water. After incubation period, 2mL of the above incubated solution was added to 2mL of Greiss reagent and incubated at room temperature for a period of 30minutes. Scavengers of Nitric oxide compete with oxygen, leading to reduced production of nitrite ion, a pink coloured chromophore is formed. The absorbance of these solution was measured at 540nm against the corresponding blank solutions. Percentage inhibition was calculated as:

\[
\text{NO scavenging activity (\%)} = (A₀-A₁)/A₀ \times 10, \text{where } A₀ \text{ is the absorbance of the control, and } A₁ \text{ is the absorbance of the sample.}
\]

The effect of extract on lipid peroxidation inhibition was determined by the ammonium thiocyanate method Lee (2002). The principle is based on the measure of the absorbance of red colour at 500nm which decreases in the presence of antioxidants. Different concentration (0.2-6mg/ml) of the extract (0.5ml) were mixed with 0.2ml of diluted linoleic acid (25mg/ml in 99% ethanol) and 0.4ml of 50 mm phosphate buffer (pH 7.4.). After 15minutes of incubation at 40°C, an aliquot (0.1ml) from the reaction mixture was mixed with reaction solution containing 3ml of 70% ethanol, 0.1mL of ammonium thiocyanate (30mg/ml in distilled water) and 50µL of ferrous chloride (2.45 mg/ml in 3.5% hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3minutes. The absorbance was then measured at 500nm. Linoleic acid emulsion without extract served as control and vitamin C (0.2- 1mg/ ml) was used as standard control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

\[
\text{Anti-lipid peroxidation activity (\%)} = (A₀-A₁)/A₀ \times 100.
\]

Where: A₀ is the absorbance of control and A₁ absorbance of the sample extract. IC₅₀ values denote the concentration of sample which is required to inhibit 50% of Linoleic acid oxidation Data are reported as mean ± standard error of mean (SEM) and were analyzed statistically using one-way analysis of variance followed by student’s t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups using Microsoft Excel windows. A significant change was considered acceptable at P<0.001 and 0.05.

Results and Discussion

Most natural products are produced by plants and many of these are involved in plant defense. The antioxidant activity of Achyranthes aspera leaves was investigated as well as its antidiabetic effect. Oxidation reactions are necessary part of life, unfortunately they can also be damaging because of the production of reactive oxygen species (ROS). In the past few years, there has been growing interest in the involvement of ROS in several pathological situations. ROS are by products of basic metabolic processes, immune reaction against pathogens, air pollution, tobacco smoke, herbicides, and pesticides. In biological systems, phenolic compounds and flavonoids are associated with scavenging ROS. DPPH is considered as a
stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm and also by the colour change from purple to yellow, which is induced by anti-oxidants. The degree of discoloration indicates the scavenging potential of the antioxidant compound in the extracts. The higher burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body (Szkudelski T. 2001). This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases such as atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases, (Domínguez et al 2008).

The radical scavenging activity of extract of Achyranthes aspera was estimated by comparing the percentage of inhibition of formation of DPPH radicals by the extract and that of ascorbic acid. Table 1 DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The Table 1 shows the antioxidant activities of the methanol, using the DPPH radical scavenging; 62.25-500 mg/ml extracts produced moderate to high DPPH scavenging activity in the plants. The highest DPPH scavenging activity was observed in Achyranthes aspera (92.40 %). Based on the results obtained, the methanol extracts which are more polar solvent extracts, were more effective antioxidants. The DPPH method was evidently introduced nearly 50 years ago by Böhm et al and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant capacity. The parameter IC50 (~efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). In the DPPH radical scavenging assay methanolic extract exhibited antioxidant activity as shown in the Table 1 Then concentrations of this active sample were used to calculate IC50 values which were found to be significant as 28.23±0.05, 64.07±2.60, 82.50±6.67, 92.40±0.46 mg/ml respectively, as compared to ascorbic acid (99.20±0.40).

Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine, (Baynes and Thorpe 2009, Auddy et al 2003) The quantitative determination of ascorbic acid in plant extracts shows that they are good source of ascorbic acid. High quantity of ascorbic acid was found to be 92.20±0.40. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthesis (NOS), which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants. In order to evaluate the antioxidant potency through NO scavenging by the test samples, the change of optical density of NO was monitored. Table 1.2 shows NO scavenging activity of the plant extract. It is to be noted that Achyranthes aspera has inhibition of plant extracts to be 95.77 ± 1.29 while ascorbic acid has shown 99.40±0.10 inhibition of NO. The IC50 value of the extract was found to be 123mg/ml as shown in Table 2.

Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radicals-induced lipid peroxidation has gained much importance because of its involvement in several pathological conditions such as aging, wound healing, oxygen toxicity, liver disorders, inflammations etc. The results of this investigation showed that the extract inhibited lipid peroxidation in a dose dependent manner (Table 3). Anti-lipid peroxidation activity of Achyranthes aspera is significant 99.23±0.21 as compared to ascorbic acid as 99.87±0.05. The plant kingdom represents a rich store house of organic compounds, of which have been used for medicinal and other purposes. Antioxidant activity of Methanol extract of the plant was shown in Table 3 In methanol extract, the plant exhibited very high antioxidant activity which was closer to ascorbic acid (99%). The antioxidant activities of methanol extract were above 90%. The plant extract constitutes have been reported to have free radical scavenging activity (Ceriello and Mortz 2004). Interestingly, flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals and inhibitors of lipid peroxidation (Syyanne and Taskinen, 2007). The medicinal properties of folk plants are mainly attributed to the presence of phenolic compounds mostly flavonoids, phenolic acids and antioxidant micronutrients (Steinberg, D. 2002). Therefore it is pertinent to explore this herb and plant which is capable of producing naturally occurring antioxidants.

Diabetes mellitus was induced by intra peritoneal injection of alloxan to all the group of animals that caused severe diabetes in rats. Table 4 shows the levels of blood sugar, and plasma insulin in diabetes induced untreated as well as treated animals. Effect of aqueous extract of Achyranthes aspera Linn alloxan induced diabetic rats was evident from the results of group 1 and 11; 112±3.54 and 112.5±10.61 (500mg/kg and 250mg/kg) respectively. A. aspera L. at a dose of 500mg/kg produced a significant 112.5± 3.54 (p<0.05) fall in blood sugar level in diabetic rats. Animals treated with standard drug also showed a significant reduction 175±4.24 in blood glucose level compared to group 1 (p<0.05). The level of liver glycogen was reduced in alloxanized diabetic group IV rats.

The concentration of fasting blood glucose was increased in the alloxan induced diabetic rats. Alloxan is known to destroy the β-cells of islets of pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose. However, this parameter was decreased significantly in the diabetic rats placed on Achyranthes aspera extract. This agrees with earlier works done by Frei,B et al 2008 who reported a hypoglycemic action of the pectin present in the decoction of Achyranthes aspera. The alloxan induced diabetic rats showed a marked loss in body weight (Table 4). This would be expected as one of the symptoms of diabetes is body weight loss. With destruction of pancreatic cells by alloxan, there’s deficiency of insulin leading to increased synthesis of ketone bodies which are excreted in urine. With increased synthesis of ketone bodies coupled with increased lipolysis leads to a severe body weight loss. However, the diabetic rats showed a remarkable gain in weight when placed on Achyranthes aspera.
In light of our results, our study indicates that Achyranthes aspera leaves extracts have antidiabetic activity. A. aspera exhibited significant anti-hyperglycemic activities in alloxan induced hyperglycemic rats without significant change in body weight. According to these results, A. aspera could be supplement, as an antioxidant therapy, and may be beneficial for correcting the hyperglycemia and preventing diabetic complications.

Currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs.

Conclusion and Recommendations

The herbals occupied a distinct place in the life right from the primitive period till date and provided information on the use of plants products and products as medicine. The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents and dates back antiquity. It is seen from the introduction that Achyranthes aspera is a very important plant for its large number of medicinal properties as well as medicinally important chemicals like achyranthine, ecdysterone, betaine, etc. The plant shows many pharmacological activities like cardiovascular, nephroprotective, hypoglycemic, analgesic and antipyretic. Many traditional uses are also reported like antiperiodic, purgative and laxative, in various types of gastric disorders and in body pain which are being studied till today and further research has to be done. The experimental evidence obtained in the present laboratory animal study indicates that the ethanol leaf of Achyranthes aspera possess antioxidant properties for curbing oxidative stress complications. The efficacy of extracts can be attributed to the presence of biologically active components which may be worth further investigating and elucidating.

References


### Tables

**Table 1:** DPPH Radical Scavenging Extract and Vitamin C.

<table>
<thead>
<tr>
<th>Concentration (Mg/MI)</th>
<th>Mean Of Inhibition ± SD (%)</th>
<th>IC(_{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.25</td>
<td>23.83 ± 7.05</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>64.07 ± 2.60</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>82.50 ± 6.87</td>
<td>108mg/ml</td>
</tr>
<tr>
<td>500</td>
<td>92.40 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Vitamin C 300mg</td>
<td>99.20 ± 0.40</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Nitric oxide inhibition activity.

<table>
<thead>
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<th>Concentration (Mg/MI)</th>
<th>Mean Of Inhibition ± SD (%)</th>
<th>IC(_{50})</th>
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</thead>
<tbody>
<tr>
<td>62.25</td>
<td>26.77 ± 1.80</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>44.47 ± 1.99</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>84.67 ± 9.19</td>
<td>123mg/ml</td>
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<tr>
<td>500</td>
<td>95.77 ± 1.29</td>
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<tr>
<td>Vitamin C 300mg</td>
<td>99.40 ± 0.10</td>
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**Table 3:** Anti-lipid peroxidation activity.

<table>
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<th>IC(_{50})</th>
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<tr>
<td>62.25</td>
<td>16.77 ± 1.40</td>
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<tr>
<td>125</td>
<td>67.43 ± 14.01</td>
<td></td>
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<tr>
<td>250</td>
<td>93.60 ± 1.23</td>
<td>109mg/ml</td>
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<tr>
<td>500</td>
<td>99.23 ± 0.21</td>
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</tr>
<tr>
<td>Vitamin C 300mg</td>
<td>99.87 ± 0.05</td>
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</tbody>
</table>

**Table 4:** Fasting blood sugar result for anti-diabetic effect of *A. aspera*.

<table>
<thead>
<tr>
<th>Dose Administration</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
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</thead>
<tbody>
<tr>
<td>500mg</td>
<td>0.10</td>
<td>1.28</td>
<td>1.09</td>
<td>0.86</td>
<td>0.78</td>
<td>0.88</td>
<td>0.78</td>
<td>0.68</td>
<td>0.78</td>
<td>0.68</td>
</tr>
<tr>
<td>250mg</td>
<td>0.10</td>
<td>1.28</td>
<td>1.09</td>
<td>0.86</td>
<td>0.78</td>
<td>0.88</td>
<td>0.78</td>
<td>0.68</td>
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<td>0.68</td>
</tr>
<tr>
<td>125mg</td>
<td>0.10</td>
<td>1.28</td>
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<td>0.78</td>
<td>0.88</td>
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<td>0.68</td>
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<td>0.68</td>
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<tr>
<td>62.25mg</td>
<td>0.10</td>
<td>1.28</td>
<td>1.09</td>
<td>0.86</td>
<td>0.78</td>
<td>0.88</td>
<td>0.78</td>
<td>0.68</td>
<td>0.78</td>
<td>0.68</td>
</tr>
<tr>
<td>Pos. Ctrl</td>
<td>143±</td>
<td>0.90</td>
<td>147±</td>
<td>7.78</td>
<td>151±</td>
<td>6.36</td>
<td>142±</td>
<td>10.6</td>
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<tr>
<td>Neg. Ctrl</td>
<td>171±</td>
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<td>5.66</td>
<td>156±</td>
<td>10.9</td>
<td>167±</td>
<td>2.62</td>
</tr>
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</table>
**Figures**

**Figure 1:** Fasting blood sugar for anti-diabetic effects of *A. aspera*

*Positive Control: Diabinase, Negative control: Normal saline.*