Evaluation of Aqueous Extract of *Alphonsea sclerocarpa* for *in vivo* and *in vitro* Antidiabetic Potentials in Wistar Rats

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**ABSTRACT**

The purpose of current investigation was to investigate *in vivo* and *in vitro* anti-diabetic potentials of aqueous extract of *Alphonsea sclerocarpa* leaves against alloxan induced diabetes in albino rats. Two *in vivo* and one *in vitro* methods were performed for the evaluation of aqueous extract for antidiabetic activity. For *in-vivo* evaluation, diabetes was induced in albino rats by administering a single dose of alloxan. The study was designed to test the acute effect of aqueous extract of *Alphonsea sclerocarpa* (AEAS) to reduce blood glucose in OGTT. The chronic study of 21 days was performed against diabetic rats and blood glucose was determined at 1st, 7th, 14th and 21st day. In chronic *in vivo* study, serum parameters insulin, urea, creatinine, total cholesterol, triglycerides, ALT and AST were also estimated at 21st day to determine the effects of aqueous and aqueous extracts on complications of diabetes mellitus. Glucose uptake by hemidiaphragm assay was performed to test the ability of extract to utilize glucose. In Oral Glucose Tolerance Test, standard glibenclamide and aqueous extract (200mg/kg and 400mg/kg) treated animals have shown significant reduction in blood glucose at 90 mins but at 120 mins. In chronic model the aqueous extract effectively reduced blood glucose levels (P<0.001) at 14th and 21st day of study in therapeutic groups and effect was comparable to that of standard. The extract could also significantly (P<0.001) reduce concentrations of SGOT, triglycerides, cholesterol and urea in serum and significantly (P<0.001) increased the insulin level in blood which proves beneficial effects of the extract in diabetes. The change in concentrations of SGPT and urea were less significant (P>0.01). The presence of extract in glucose uptake assay could significantly increase utilization of the glucose by rat hemidiaphragm. The aqueous extract of *Alphonsea sclerocarpa* possess significant antidiabetic properties against alloxan induced diabetic animals.

**Keywords:** Antidiabetic activity, *Alphonsea sclerocarpa*, Alloxan, Blood glucose, Insulin, Total cholesterol, Triglycerides, Creatinine, Urea, Alanine transferase and Aspartate transferase.

**INTRODUCTION**

Diabetes mellitus (DM) is a chronic metabolic disorder which results from the lack of insulin secretion in the body and leads to disturbances in carbohydrate, protein and lipid metabolism. Besides typical symptoms like hyperglycemia, weight loss, polyurea and polydypsia, Diabetes mellitus has several other symptoms that includes hyperlipidemia, which are involved in the development of micro-vascular and macrovascular complications of diabetic patient and may leads to death. The diabetes mellitus is a non-infective pathological condition which could hit the world this millennium. It is estimated that, by 2025 the half the diabetic patients worldwide will be from India and it would become “Diabetic capital of the world”.

Type II-Insulin dependent diabetes mellitus also known as non-insulin dependent diabetes mellitus, which develops in middle or later life and affects 2–6% of adults in most Western societies. Diabetes mellitus (DM) is the most common health problem of the world in the current century. Nowadays more than 366 million people suffer from DM and according to World Health Organization estimates, 552 million are expected to be affected by diabetes by 2030. Treatment of hyperglycemia in diabetes involves diet control, exercise and the use of hypoglycemic drugs (oral). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are widely prescribed even when their biologically active compounds are not understood.

The use of pharmacological and chemical agents currently available for the treatment of type II diabetes include sulfonylurea, biguanide, thiazolidinedione and α glycosidase inhibitors are known to possess several undesirable side effects and fail to significantly alter the course of diabetic complications. At present, insulin is the...
choice of drug for the treatment of insulin dependent diabetes mellitus (Type I IDDM) where as other synthetic drug like sulfonylureas and insulin sensitizers are the effective drugs for curing non-insulin dependent diabetes mellitus (Type II-NIDDM). But these drugs possess very serious and potential adverse effects like cardiotoxicity, nephrotoxicity and etc. Hence irrespective of tremendous advancements in the medical field there is no truly satisfactory drug available for the treatment of diabetes mellitus. Hence always there is scope to develop drugs from plant origin which already effectively used in Indian traditional system like Ayurveda for treatment of diabetes mellitus and WHO always encourages the research activities from natural sources to prevent the high prevalence of diabetes as well as its long-term complications. Since ancient times herbal remedies have been used for the treatment of diabetes mellitus. About 90% of the world population in rural areas of developing countries relies solely on traditional medicines for their primary health care. The use of foods and medicinal plants to improve health is nearly as old as humanity. The Alphonsea is a genus of plant which is of Indian origin. The various species of Alphonsea are medicinally important and have been proved for their several pharmacological activities. A number of Alphonsea species are used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine (TCM) for various purposes such as diabetes, hepatitis, kidney disease, cancer, menstrual irregularities especially amongst people where these species grow. These uses, however, originated and are most widely found in the Middle East. The Alphonsea sclerocarpa of family Annonnacea medicinally important plants belongs to the same genus. Their extracts scientifically proved for many pharmacological activities in animal models. The Alphonsea sclerocarpa was extensively used in folklore and traditional medicine for the management liver toxicity and diabetes but lacks the scientific evidence for the same. Hence the present research work aimed to explore anti-diabetic potentials of leaves of Alphonsea sclerocarpa.

MATERIALS AND METHODS

Plant material

The leaves of Alphonsea sclerocarpa had been collected from Sri Venkateshwara University, Tirupati, India and shade dried. The leaves were identified, collected and authenticated by Dr. Madhava chetty Asst.Prof. Dept. of Botany and specimen herbarium were preserved at institute herbarium library. The authenticated leaves were separated from other plant parts, cleaned, washed and dried for further use.

Preparation of aqueous extract of Alphonsea sclerocarpa

The shade dried leaves were pulverized into powder and sieved through No. 22 mesh. About 350 g (appx.) of coarse powder was defatted using petroleum ether and the marc leftover was extracted using aqueous in soxhlet apparatus.

Preliminary phytochemical investigation of aqueous extract of Alphonsea sclerocarpa

The preliminary phytochemical investigation for the aqueous extract of Alphonsea sclerocarpa had been conducted as per procedure prescribed by Khandelwal.

Drugs and chemicals

The alloxan was procured from Sigma Aldrich and all the chemical and reagents used in the present investigation were of analytical grade and procured SD Fine chemicals, Bangalore.

Animals

The healthy albino wistar male rats were procured from Sri Venkateswara Enterprises, Bangalore housed under standard conditions of temperature (22 ± 10C), relative humidity (55 ± 10%), 12 hr light/dark cycles and fed with standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Acute Oral Toxicity Studies

The OECD guidelines 423 (up and down procedure) were used to determine acute oral toxicity for aqueous extract of Alphonsea sclerocarpa. A starting dose used was 2000 mg/kg body weight p.o. of extract (AEAS was administered to 3 male rats, observed for 14 days. The experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more and observed for 14 days.

Evaluation of in vivo antidiabetic activity

The antidiabetic activity of aqueous extract was evaluated against alloxan induced diabetes in rats model by following two methods.

- Oral Glucose Tolerance Test
- Chronic study

Induction of diabetes mellitus in experimental animals

Type II diabetes mellitus (NIDDM) was induced in overnight fasted adult male Wistar albino rats weighing 150–200 g by a single intraperitoneal injection of alloxan monohydrate at 120mg/kg,b.w. Hyperglycemia is confirmed by the elevated glucose levels determined at 72 hrs. Animals with blood glucose level more than 150 mg/dl were considered as diabetic animals.
Oral Glucose Tolerance Test

**Group classification**

The experimental design for the present research work consists of two different sets of animals for both OGTT and chronic anti-diabetic activity study. The group classification and treatment protocol was as follows.

**Table 1: Group classification and treatment protocol**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>Treated with normal saline 5ml/kg i.p</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>Treated with alloxan (120 mg/kg i.p) and Vehicle</td>
</tr>
<tr>
<td>III</td>
<td>Standard Control</td>
<td>Treated with alloxan (120 mg/kg i.p) and Glibenclamide 5mg/kg.</td>
</tr>
<tr>
<td>IV</td>
<td>AEAS-100mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and low dose (100mg/kg, p.o) of aqueous extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
<tr>
<td>V</td>
<td>AEAS-200mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and medium dose (200mg/kg., p.o) of aqueous extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
<tr>
<td>VI</td>
<td>AEAS-400mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and high dose (400mg/kg., p.o) of aqueous extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
</tbody>
</table>

Glucose uptake by isolated rat hemidiaphragm

The utilization of glucose by skeletal muscle of rat(hemidiaphragm) was assed according to methods described in previous investigations. The study consisting of four categories, with each group containing 6 graded test tubes, were regarded as follows:

- **Category I:** Consists of 2 mL of 2% glucose in Tyrode solution.
- **Category II:** Consists of 2 mL of 2% glucose in Tyrode solution and regular insulin suspension.
- **Category III:** Consists of 2 mL of 2% glucose in Tyrode solution and 1.38 mL of AEAS (0.1% v/v).
- **Category IV:** Consists of mL of 2% glucose in Tyrode solution and regular insulin (0.62 mL of 0.4 U/mL) solution and 1.38 mL of AEAS (0.1% v/v).

The quantities of all the assay tubes were make up to 4 mL individually by mixing distilled water to make up the total volume of the assay tubes. A total of healthy albino rats of wistar species were kept fasting for whole night and sacrificed under light anesthesia. The diaphragms of experimental animals were quickly cutted with little damage and splitted into 2 equal halves. For the same set of study, two diaphragms from the same animal were not used. About xix diaphragms were utilized in every category of study. The collected skeletal muscles (diaphragm) were kept in assay tubes and incubated at 37°C for about 30 minutes in an atmosphere constitutes 100% oxygen and were shuddered at a speed of 140 CPM. The amount of utilization of glucose per every gram of tissue was determined as the difference between the concentrations of starting and final glucose in the incubated medium.
Statistical Analysis

The data obtained from the present investigation were analyzed by ANOVA followed by post hoc Dunnet’s t-test with the help of Graphpad prism5 software. All the values were shown as mean ± standard error of mean (S.E.M.).

RESULTS & DISCUSSION

Preliminary phytochemical study

The percentage yield of the AEAS was found to be 9.22% w/w. The preliminary phyto-chemical investigation of the aqueous extract of Alphonsea sclerocarpa reveals the presence of alkaloids, glycosides, poly phenols, flavonoids, tannins, steroids, and carbohydrates.

Acute toxicity studies

The aqueous extract of Alphonsea sclerocarpa was safe up to dose of 2000 mg kg\(^{-1}\) b.w. and caused neither mortality nor any signs of clinical abnormality in the tested animals during the observation period of 14 days after administration of highest dose. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, no changes were observed for 14 days. As per the results obtained in acute oral toxicity study doses were selected as 100, 200 and 400mg/kg on the ratio 1/20\(^{th}\), 1/10\(^{th}\) and 1/5\(^{th}\) respectively.

Evaluation of in vivo antidiabetic activity

Diabetes mellitus is a metabolic, multifactorial and devitalizing disease with increasing occurrence in the entire world. which may lead to various complications such as multi organ failures, peripheral neuropathy, retinopathy, nephropathy, hyperlipidemia and various cardiovascular disorders. Alloxan potent diabetogenic chemically a cyclic urea analogue, which specifically kills β-cells of Langerhans of pancreas that generates insulin free radically mediated destruction when given to rats can produces diabetes mellitus. Hence alloxan was reported as a potent diabetes causing agent and has been extensively given to experimental rats for the production of diabetes laboratory.

Oral Glucose Tolerance Test

In acute study of OGTT, diabetic control rats treated with vehicle have exhibited significant increase in plasma glucose range throughout investigation period when collate to normal group of rats. But administration of glibenclamide and AEAS (at 200 and 400mg/kg) could capable to reduce blood glucose significantly (P<0.001) in therapeutic animals by improving the utilization of oral glucose after 60 and 120 mins. The results of OGTT are given in Table No 2.

In the in vivo study to evaluate the acute effect of AEAS, Oral Glucose Tolerance Test was performed to test the ability of the body to utilize oral glucose load in presence of aqueous extract of Alphonsea sclerocarpa (AEAS) in diabetic animals. In diabetic control group of animals, the blood glucose was significantly elevated at all intervals indicating the reduced ability of the system to utilize the glucose whereas the blood glucose had fall down below 100mg/dl in normal group of animals since the ability of the body was proper. Therapeutic groups treated with low and medium doses of AEAS significantly decreased blood glucose concentration at 90 mins and 120 mins interval indicates property of AEAS to enhance the utilization of glucose by living system and this effect was comparable to the reference standard drug glibenclamide.

Chronic antidiabetic study

In long standing antidiabetic test, the blood glucose level was significantly (P<0.001) elevated in disease (diabetic) alone rats when compared to normal group due to the induction of diabetes. While administration of glibenclamide and AEAS at 200mg/kg and 400mg/kg could significantly (P<0.001) decreased ranges of glucose in blood compare to diabetic control group at 14th and 21st day of the study [Table No .3].

The chronic in vivo study was designed to examine the long term consequences of aqueous extract against alloxan produced diabetes in albino wistar rats. The blood glucose range in experimental animals was assessed at every 7 day interval of the investigation to test the ability of the AEAS in removing glucose from bloods in diabetic animals and furthermore, insulin, total cholesterol, triglycerides, urea, creatinine, ALT and AST in serum at21st day of the study. In chronic study of 21 days, there was significant rise in blood glucose range observed in disease control animals throughout study due to the destruction of β-cells of pancreas and impairment in insulin secretion. But in animal groups treated with AEAS (at 200 and 400mg/kg), blood glucose significantly declined was at 14th and 21st day of the study which was witnessed by the enhancement in insulin secretion. This clearly indicates the potential of the TPME to reverse the pancreatic β cell damage.

The significant (P<0.001) decline of concentration of serum insulin was found in vehicle control group compare to normal group due to the treatment of alloxan. In animals administered with glibenclamide and AEAS (200mg/kg and 400mg/kg) there was considerable (P<0.001) elevation in plasma insulin quantities when compared to diabetic control group and the results were almost similar to that of normal animals [Table No .4].

The total serum cholesterol, triglycerides, urea and creatinine concentrations in the blood sample were significantly (P<0.01) elevated in diabetic alone animals collate to normal group of animals. But decline in the concentrations of serum total cholesterol, triglycerides, urea and Creatinine was found in glibenclamide and AEAS (200mg/kg 400mg/kg) pretreated rats when compare to disease control rats [Table No .4].

It is found that there is no significant (p>0.01) alterations in AST and ALT levels in diabetic alone compare to normal
animals and also no significant change was found in therapeutic group given with reference standard and AEAS when compared to rats of vehicle control group. [Table No: 4].

Table 3: Effect of aqueous and aqueous extracts of *Alphonsea sclerocarpa* on blood glucose against OGTT in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of Blood Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Mins</td>
</tr>
<tr>
<td>Normal Control</td>
<td>82.5±1.56</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>173.5***±3.9</td>
</tr>
<tr>
<td>Standard (Glibenclamide 5mg/kg)</td>
<td>176.3±3.4</td>
</tr>
<tr>
<td>AEAS 100 mg/kg</td>
<td>72.7±3.6</td>
</tr>
<tr>
<td>AEAS 200 mg/kg</td>
<td>174.8±4.37</td>
</tr>
<tr>
<td>AEAS 400 mg/kg</td>
<td>172.2±4.23</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance; "p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control. *p>0.05, ** p<0.01, ***p<0.001 normal control vs positive control.

Table 4: Effect of aqueous and aqueous extracts of *Alphonsea sclerocarpa* on blood glucose against chronic study in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of Blood Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Normal Control</td>
<td>82.5±1.56</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>173.5***±3.9</td>
</tr>
<tr>
<td>Standard (Glibenclamide 5mg/kg)</td>
<td>176.3±3.4</td>
</tr>
<tr>
<td>AEAS 100 mg/kg</td>
<td>246.5±4.65</td>
</tr>
<tr>
<td>AEAS 200 mg/kg</td>
<td>233.2±5.04</td>
</tr>
<tr>
<td>AEAS 400 mg/kg</td>
<td>241.7±3.904</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance; "p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control. *p>0.05, ** p<0.01, ***p<0.001 normal control vs positive control.

Along with other risk factors, secondary hyperlipidemia is one of the major cause of increased incidence of coronary atherosclerosis which is significantly observed in people with prolonged diabetes mellitus. Hyperlipidemia is impediment in metabolism which is characterized by the enhanced plasma cholesterol and triglycerides [24,25,26,27]. In the present study, the aqueous extract of *Alphonsea sclerocarpa* showed significant elevation of serum cholesterol and triglycerides when compared with untreated control group. The glucose utilization by rat skeletal muscle was significantly enhanced in diabetic alone animals due to renal complication in diabetes mellitus. The amount of serum creatinine and urea was significantly increased in diabetic alone animals due to renal complication in diabetes mellitus. The therapeutic administration of AEAS (200mg/kg and 400mg/kg) in diabetic control animals indicates the potential of the AEAS to reverse renal complication in diabetes mellitus.

It is well known that there is close connection between liver disease and diabetes, the general pervasiveness being considerably larger than that anticipated by a chance relation of two more general diseases[24-26]. In the present study, no significant changes or rise of the liver enzymes AST and ALT were observed in diabetic alone animals when compared to normal group of animals. The concentrations of ALT and AST in therapeutic groups were also normal.

Glucose uptake by isolated rat hemidiaphragm

In the present study, the aqueous extract of *Alphonsea sclerocarpa* significantly increased utilization of glucose by rat hemidiaphragm and the effect was comparable to standard agent Insulin. The combination of AEAS with insulin has shown synergistic property. The results clearly indicate that administration of insulin and AEAS alone for 30 minutes caused a significant enhancement in glucose absorption by 3.37- and 2.80- times, respectively. Addition of both insulin and AEAS to the incubation media exhibited the rate by 3.55-times, an elevation of utilization of glucose hemidiaphragm of rat when compared with untreated control animals but there was no much significant elevation compared insulin alone treated group [Table No 5]. The glucose utilization by rat skeletal muscle was considerably large in all the categories examined when collate with the vehicle control.
Table 5: Effect of aqueous and aqueous extracts of *Alphonsea sclerocarpa* on blood glucose against OGTT in alloxan induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin (IU/L)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>137.7± 2.74</td>
<td>81.68± 2.72</td>
<td>106.8± 2.73</td>
<td>0.5708± 0.03</td>
<td>32.73± 0.81</td>
<td>64.92± 2.73</td>
<td>131.4± 2.66</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>79.83**± 1.82</td>
<td>110.1**± 3.18</td>
<td>133.5**± 2.61</td>
<td>1.531***± 0.1</td>
<td>74.19***± 2.75</td>
<td>63.98± 2.78</td>
<td>135.6± 2.36</td>
</tr>
<tr>
<td>Glibenclamide (5mg/kg)</td>
<td>108.3**± 3.26</td>
<td>81.25*± 3.16</td>
<td>102.6**± 3.84</td>
<td>0.70***± 0.07</td>
<td>35.85***± 1.9</td>
<td>64.90± 1.78</td>
<td>128.5± 1.97</td>
</tr>
<tr>
<td>AEAS 100 mg/kg</td>
<td>75.83± 9.53</td>
<td>103.2± 4.31</td>
<td>33.1± 0.73</td>
<td>1.28± 0.066</td>
<td>67.13± 1.28</td>
<td>65.74± 2.87</td>
<td>133.5± 2.44</td>
</tr>
<tr>
<td>AEAS 200 mg/kg</td>
<td>113.5**± 1.57</td>
<td>87.76***±8.67</td>
<td>113.7**± 2.19</td>
<td>0.9685**± 0.05</td>
<td>51.57***± 2.08</td>
<td>64.79± 2.50</td>
<td>131.8± 2.27</td>
</tr>
<tr>
<td>AEAS 400 mg/kg</td>
<td>131.7***± 5.51</td>
<td>86.47***±2.94</td>
<td>104.1***± 3.49</td>
<td>0.6737***± 0.09</td>
<td>33.88***± 1.91</td>
<td>64.69± 1.68</td>
<td>125.7± 3.35</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance.; ** p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control.

Skeletal muscle comprises about 30-40% of the total quantity of body and hence it can be one of the most major target tissues for the activity of insulin which enhances the utilization of glucose at the peripheral level. It is a well understood that insulin and anti-diabetic drugs stimulate glucose utilization by peripheral cells and tissues. Other major finding of the present study is that AEAS have significant action similar to insulin as witnessed by the stimulation of glucose utilization from the rat’s hemidiaphragm, which constitutes muscle tissue that are essential tissues of insulin regulated glucose discharge. The AEAS considerably enhanced the uptake of glucose by isolated rats muscle hemidiaphragm and is observed to be less potant than insulin. It seems that AEAS has action on peripheral tissues and results of normal group of glucose utilization by rat peripheral tissue corresponds with those of earlier findings.

Table 6: Effect of AEAS on glucose uptake by isolated rat hemi-diaphragm

<table>
<thead>
<tr>
<th>S. No</th>
<th>Glucose uptake for 30 mins (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.24±1.52</td>
</tr>
<tr>
<td>Insulin</td>
<td>272.15±2.65**</td>
</tr>
<tr>
<td>AEAS</td>
<td>219.54±1.9**</td>
</tr>
<tr>
<td>AEAS + Insulin</td>
<td>275.42±2.93**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6); ** p < 0.01 as compared with control

In spite there is no clear specific mechanism of alloxan responsible for pancreatic damage understood, investigations propose that the alloxan destroys pancreatic β cells due to its free radical nature which followed by absolute insulin deficiency and diabetes mellitus [29,30]. The previous researches conducted have suggests that antioxidant activity can be one of the possible mechanism of action for antidiabetic activity that protects pancreatic cells against oxidative damage [31]. Hence further study can be performed to explore antioxidant activity of AEAS to determine its ability to reduce reducing insulin resistance which is also important mechanism required for antidiabetic activity.

In the current in vivo assay the aqueous extract had been effective to stimulate insulin secretion and to regulate the normal glucose level in the therapeutic groups. The study should be conducted to determine the antioxidant properties of AEAS which is possible mechanism of action in the present study that can defend pancreatic cells against alloxan mediated damage and normalize the insulin release. In in vitro findings the AEAS exhibited its potency to counter insulin resistance by increasing the utilization of glucose by peripheral tissues.

**CONCLUSION**

The aqueous extract of ariel parts of *Alphonsea sclerocarpa* possess significant in vivo anti-diabetic activity against alloxan induced diabetic animal model. The results acquired from the present study also proposes that aqueous extract of *Alphonsea sclerocarpa* also significantly increase utilization of glucose by skeletal muscle. But further examination is necessary to isolate and estimate the specific components present in aqueous extract of *Alphonsea sclerocarpa* that may be responsible for these beneficial properties to improve the health conditions connected with diabetes mellitus.

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REFERENCES


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Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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