In Vivo and In Vitro Evaluation of Ethanol Extract of Alphonsea sclerocarpa for Antidiabetic Properties in Wistar Rats

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Submitted: 22 January 2022
Accepted: 27 January 2022
Published: 28 February 2022

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

ABSTRACT

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

Diabetes mellitus (DM) is a chronic metabolic disorder that 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, polyuria, and polydipsia, Diabetes mellitus has several other symptoms that include hyperlipidemia, which is involved in the development of microvascular and macrovascular complications of the diabetic patient and may lead to death. Diabetes mellitus is a non-infective pathological condition that could hit the world this millennium. It is estimated that by 2025 half the diabetic patients worldwide will be from India and it would become the “Diabetic capital of the world [1,2].

Type II-Insulin dependent diabetes mellitus also known as non-insulin-dependent diabetes mellitus, develops in middle or later life and affects 2–6% of adults in most Western societies [3]. 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 [4].

The use of pharmacological and chemical agents currently available for the treatment of type II diabetes including 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) whereas 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, etc [5]. 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 the Indian traditional system like Ayurveda for the treatment of diabetes mellitus and WHO always encourages research activities from natural sources to prevent the high prevalence of diabetes as well as its long term complications [4]. 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 [5,6].

The use of foods and medicinal plants to improve health is nearly as old as humanity. The *Alphonsea* is a genus of the plant which is of Indian origin. The various species of *Alphonsea* are medicinally important and have been proved for their several pharmacological activities [7]. Several *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 [8]. The *Alphonsea sclerocarpa* of the family Annonnacea medicinally important plants belongs to the same genus [7]. Their extracts are scientifically proved for many pharmacological activities in animal models. The *Alphonsea sclerocarpa* was extensively used in folklore and traditional medicine for the management of liver toxicity and diabetes but lacks the scientific evidence for the same. Hence the present research work aimed to explore the 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 the institute herbarium library. The authenticated leaves were separated from other plant parts, cleaned, washed, and dried for further use.

*Preparation of ethanol extract of *Alphonsea sclerocarpa***

The shade dried leaves were pulverized into powder and sieved through No. 22 mesh. About 350 g (aprx.) of coarse powder was defatted using petroleum ether and the marc leftover was extracted using ethanol in the soxhlet apparatus [9].
Preliminary phytochemical investigation of ethanol extract of *Alphonsea sclerocarpa*

The preliminary phytochemical investigation for the ethanol extract of *Alphonsea sclerocarpa* was conducted as per the procedure prescribed by Khandelwal [10].

**Drugs and chemicals**

The alloxan was procured from Sigma Aldrich and all the chemicals 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 ± 1°C), relative humidity (55 ± 10%), 12 hr light/dark cycles, and fed with a standard pellet diet (Amrut, Pranav Agro Industries Ltd., Sangli, India) and water ad libitum. After randomization into various groups and before initiation of the experiment, the rats were acclimatized for 7 days under the above said environmental conditions. The experimental protocol has been approved by the Institutional Animals Ethics Committee with permission from the Committee for 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 ethanol extract of *Alphonsea sclerocarpa*. A starting dose used was 2000 mg/kg body weight p.o. of extract (EEAS was administered to 3 male rats, observed for 14 days. The experiments were repeated with the same dose level, 2000 mg/kg body weight p.o. of extracts for 3 days more, and observed for 14 days [11].

**Evaluation of *in vivo* antidiabetic activity**

The antidiabetic activity of ethanol 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 120 mg/kg, b.w. Hyperglycemia is confirmed by the elevated glucose levels determined at 72 hrs. Animals with blood glucose levels more than 150 mg/dl were considered diabetic animals [12].

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 were as follows.

<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 5 ml/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>EEAS-100 mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and low dose (100 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
<tr>
<td>V</td>
<td>EEAS-200 mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and medium dose (200 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
<tr>
<td>VI</td>
<td>EEAS-400 mg</td>
<td>Treated with alloxan (120 mg/kg i.p) and high dose (400 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em>.</td>
</tr>
</tbody>
</table>

The oral glucose tolerance test will perform in overnight fasted (18 h) diabetic rats. Rats divided into seven groups, each consisting of six rats will be administered 0.9% (w/v) saline, diabetic control, glibenclamide 5 mg/kg, ethanol extract of *Alphonsea sclerocarpa*. Glucose (3 g/kg) will feed 30 min after the administration of extracts. A blood sample is withdrawn from the retro-orbital sinus under ether inhalation at 0, 30, 60, and 120 min of glucose administration, and glucose levels will be estimated [13-15]. using glucose oxidase–peroxidase reactive strips and a glucometer (Accuchek, Roche Diagnostics, USA).
Chronic antidiabetic study

Group classification: The group classification and treatment protocol in chronic antidiabetic activity study were as follows.

<table>
<thead>
<tr>
<th>Sl.No</th>
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<td>Treated with alloxan (120 mg/kg i.p) and Glibenclamide 5mg/kg.</td>
</tr>
<tr>
<td>IV</td>
<td>EEAS-100 mg</td>
<td>Treated with a single dose of alloxan (120 mg/kg i.p) and low dose (100 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em> for 21 days.</td>
</tr>
<tr>
<td>V</td>
<td>EEAS-200 mg</td>
<td>Treated with a single dose of alloxan (120 mg/kg i.p) and medium-dose (200 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em> for 21 days.</td>
</tr>
<tr>
<td>VI</td>
<td>EEAS-400 mg</td>
<td>Treated with a single dose of alloxan (120 mg/kg i.p) and a high dose (400 mg/kg., p.o) of ethanol extract of <em>Alphonsea sclerocarpa</em> for 21 days.</td>
</tr>
</tbody>
</table>

The experimental design for the chronic study of 21 days is as given in the above table. The chronic antidiabetic study was performed in diabetic rats. Rats are divided into nine groups, each consisting of six rats which were administered with 0.9% (w/v) saline, diabetic control, was induced by single-dose administration of alloxan (120mg/kg i.p) in all the animals except the normal group. The animals were treated with glibenclamide, ethanol extract as given in the above table daily for 21 days. Blood samples from each rat were collected on days 1st, 7th, 14th, and 21st and estimated for blood glucose. On the last day of study blood samples were estimated for serum alanine transferase (SGPT or ALT), serum aspartate transferase (SGOT or AST), cholesterol, triglycerides, urea, and insulin [13-15].

Glucose uptake by isolated rat hemidiaphragm

The utilization of glucose by the skeletal muscle of rats (hemidiaphragm) was assessed according to methods described in the previous investigations. The study consisting of four categories, with each group containing 6 graduated 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 EEAS (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 EEAS (0.1% v/v)

The quantities of all the assay tubes were made 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 the whole night and sacrificed under light anesthesia. The diaphragms of experimental animals were quickly cut with little damage and split into 2 equal halves. For the same set of studies, 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 that constitutes 100% oxygen and were shuddered at a speed of 140 CPM. The amount of utilization of glucose per gram of tissue was determined as the difference between the concentrations of starting and final glucose in the incubated medium [16,17].

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 Prism 5 software. All the values were shown as mean±standard error of the mean (S.E.M.).

RESULTS AND DISCUSSION

Preliminary phytochemical study

The percentage yield of the EEAS was found to be 9.22 % w/w. The preliminary phytochemical investigation of the ethanol extract of Alphonsea sclerocarpa reveals the presence of alkaloids, glycosides, polyphenols, flavonoids, tannins, steroids, and carbohydrates.
Acute toxicity studies

The ethanol extract of *Alphonsea sclerocarpa* was safe up to the 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 the 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 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 the acute oral toxicity study doses were selected as 100, 200, and 400 mg/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 [19, 20]. Alloxan potent diabetogenic chemically a cyclic urea analog, which specifically kills β-cells of Langerhans of the pancreas that generates insulin-free radical-mediated destruction when given to rats can produce diabetes mellitus. Hence alloxan was reported as potent diabetes causing agent and has been extensively given to experimental rats for the production of diabetes laboratory.

Oral Glucose Tolerance Test

In an acute study of OGTT, diabetic control rats treated with the vehicle have exhibited a significant increase in plasma glucose range throughout the investigation period when collate with a normal group of rats. But the administration of glibenclamide and EEAS (at 200 and 400 mg/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 1.

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

**Chronic antidiabetic study**

In the long-standing antidiabetic test, the blood glucose level was significantly (P<0.001) elevated in disease (diabetic) alone rats when compared to the normal group due to the induction of diabetes. While administration of glibenclamide and EEAS at 200 mg/kg and 400 mg/kg could significantly (P<0.001) decrease ranges of glucose in blood compared to the diabetic control group on the 14th and 21st day of the study [Table No. 2].

The chronic in vivo study was designed to examine the long-term consequences of ethanol extract against alloxan-produced diabetes in albino Wistar rats. The blood glucose range in experimental animals was assessed at every 7 days’ interval of the investigation to test the ability of the EEAS to remove glucose from the blood in diabetic animals and insulin, total cholesterol, triglycerides, urea, creatinine, ALT, and AST in serum at 21st day of the study [21, 22, 23].

In a chronic study of 21 days, there was a significant rise in blood glucose range observed in disease control animals throughout the study due to the destruction of β-cells of the pancreas and impairment in insulin secretion. But in animal groups treated with EEAS (at 200 and 400 mg/kg), blood glucose significantly declined was at the 14th and 21st day of the study which was witnessed by the enhancement in insulin secretion. This indicates the potential of the TPME to reverse the pancreatic β cell damage.

A significant (P<0.001) decline of concentration of serum insulin was found in the vehicle control group compared to the normal group due to the treatment of alloxan. In animals administered with glibenclamide and EEAS (200 mg/kg and 400 mg/kg) there was considerable (P<0.001) elevation in plasma insulin quantities when compared to the diabetic control group and the results were almost similar to that of normal animals [Table No. 3].

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 the normal group of animals. But the decline in the concentrations of serum total cholesterol,
triglycerides, urea, and Creatinine was found in glibenclamide and EEAS (200 mg/kg 400 mg/kg) pretreated rats when compared to disease control rats [Table No. 3].

It is found that there are no significant (p>0.01) alterations in AST and ALT levels in diabetic alone compared to normal animals and also no significant change was found in the therapeutic group given with reference standard and EEAS when compared to rats of the vehicle control group. [Table No. 3].

Table No 1: Effect of ethanol and ethanol 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 5 mg/kg)</td>
<td>176.3±3.4</td>
</tr>
<tr>
<td>EEAS 100 mg/kg</td>
<td>73.2±4.08</td>
</tr>
<tr>
<td>EEAS 200 mg/kg</td>
<td>76.3±2.63</td>
</tr>
<tr>
<td>EEAS 400 mg/kg</td>
<td>76.5±4.193</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

\( ^{ns} p>0.05, \ ^{*} p<0.05, \ ^{**} p<0.01, \ ^{***} p<0.001 \) vs diabetic control.

\( ^{ns} p>0.05, \ ^{+} p<0.05, \ ^{++} p<0.01, \ ^{+++} p<0.001 \) normal control vs positive control.
Table No 2: Effect of ethanol and ethanol 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>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td></td>
<td>82.5±1.56</td>
<td>47.8±3.07</td>
<td>30.8±2.24</td>
<td>107.5±1.65</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td></td>
<td>173.5+++±3.9</td>
<td>282.8+++±3.43</td>
<td>253.8+++±2.7</td>
<td>30.7+++±3.78</td>
</tr>
<tr>
<td>Standard (Glibenclamide 5 mg/kg)</td>
<td></td>
<td>176.3±3.4</td>
<td>280.8±2.9</td>
<td>198.0***±3.7</td>
<td>160.5***±5.025</td>
</tr>
<tr>
<td>EEAS 100 mg/kg</td>
<td></td>
<td>238.7±2.29</td>
<td>233.8±3.62</td>
<td>218.0±2.78</td>
<td>209.5±3.91</td>
</tr>
<tr>
<td>EEAS 200 mg/kg</td>
<td></td>
<td>237.3±6.24</td>
<td>223.2±3.43</td>
<td>205.7*±1.59</td>
<td>173.7***±3.21</td>
</tr>
<tr>
<td>EEAS 400 mg/kg</td>
<td></td>
<td>234.0±7.28</td>
<td>212.8±5.29</td>
<td>172.0**±4.34</td>
<td>145.2***±2.12</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

ns p>0.05, * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control.

ns p>0.05, + 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 causes of increased incidence of coronary atherosclerosis which is significantly observed in people with prolonged diabetes mellitus. Hyperlipidemia is an impediment in metabolism which is characterized by enhanced plasma cholesterol and triglycerides [24-27]. In the present study, diabetic control animals have shown significant elevation of serum cholesterol and triglycerides while in animals administered with glibenclamide and TPME (200 mg/kg and 400 mg/kg), a significant decrease was observed compared to the diabetic control group.

The amount of serum creatinine and urea was significantly enhanced in diabetic alone animals due to renal malfunction caused by hyperglycemia but their concentrations were significantly declined due to the administration of EEAS (200 mg/kg and 400 mg/kg) in therapeutic animals indicates the potential of the EEAS to reverse renal complication in diabetes mellitus.

It is well known that there is a clear 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]. But 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 the normal group of animals. The concentrations of ALT and AST in therapeutic groups were also normal.

Table No 3: Effect of ethanol and ethanol extracts of *Alphonsea sclerocarpa* on blood glucose against OGTT in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin (IU/L)</td>
</tr>
<tr>
<td>Normal Control</td>
<td>137.7±2.74</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>79.83++±1.82</td>
</tr>
<tr>
<td>Glibenclamide (5 mg/kg)</td>
<td>108.3***±3.26</td>
</tr>
<tr>
<td>EEAS 100 mg/kg</td>
<td>94.67±2.06</td>
</tr>
<tr>
<td>EEAS 200 mg/kg</td>
<td>111.8***±2.21</td>
</tr>
<tr>
<td>EEAS 400 mg/kg</td>
<td>129.5***±4.97</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M, n=6 symbols represent statistical significance.

ns p>0.05,  * p<0.05, ** p<0.01, ***p<0.001 vs diabetic control.

ns p>0.05, + p<0.05, ++ p<0.01, +++p<0.001 normal control vs positive control.

**Glucose uptake by isolated rat hemidiaphragm**

In the present study, the ethanol extract of *Alphonsea sclerocarpa* significantly increased utilization of glucose by rat hemidiaphragm and the effect was comparable to standard agent Insulin. The combination of EEAS with insulin has shown the synergistic property. The results clearly indicate that administration of insulin and EEAS alone for 30 minutes caused a significant enhancement in glucose absorption by 3.37- and 2.80- times, respectively. The
addition of both insulin and EEAS to the incubation media exhibited the rate by 3.55-times, an
elevation of the utilization of glucose hemidiaphragm of rat when compared with untreated
control animals but there was not much significant elevation compared to insulin alone
treated group [Table No 4]. The glucose utilization by rat skeletal muscle was considerably
large in all the categories examined when collating with the vehicle control.

Skeletal muscle comprises about 30-40% of the total quantity of the 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 well understood that insulin and anti-diabetic drugs
stimulate glucose utilization by peripheral cells and tissues. Another major finding of the
present study is that EEAS have significant action similar to insulin as witnessed by the
stimulation of glucose utilization from the rat’s hemidiaphragm, which constitutes muscle
tissue that is essential tissue of insulin-regulated glucose discharge. The EEAS considerably
enhanced the uptake of glucose by isolated rats muscle hemidiaphragm and is observed to be
less potent than insulin. It seems that EEAS has an action on peripheral tissues and the results
of a normal group of glucose utilization by rat peripheral tissue correspond with those of
earlier findings [28].

Table No 4: Effect of EEAS on glucose uptake by isolated rat hemidiaphragm

<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>EEAS</td>
<td>224.81±2.1**</td>
</tr>
<tr>
<td>EEAS + Insulin</td>
<td>285.37±3.01**</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 is 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 the antidiabetic activity that protects pancreatic cells against
oxidative damage [31]. Hence further study can be performed to explore the antioxidant
activity of EEAS to determine its ability to reduce reducing insulin resistance which is also an important mechanism required for antidiabetic activity.

In the current, in vivo assay, the ethanol extract had been effective to stimulate insulin secretion and regulating the normal glucose level in the therapeutic groups. The study should be conducted to determine the antioxidant properties of EEAS which is a 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 EEAS exhibited its potency to counter insulin resistance by increasing the utilization of glucose by peripheral tissues.

CONCLUSION

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

ACKNOWLEDGMENTS: The authors of the manuscript are thankful to the Principal and Management of OPJS University, Churu, for providing facilities to conduct this research work.

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