Age related efficacy of ethanolic extract of aloe vera gel in alloxan induced young, adult and old male mice

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ABSTRACT

Diabetes mellitus is one of the most common serious metabolic disorder that is considered to be one of the five leading causes of death in the world. Diabetes is characterized by absolute or relative deficiencies in insulin secretion which associate with chronic hyperglycemia. Traditional plant medicine might offer a natural key to diabetic complications. Aloe vera is an herb which is distributed throughout the world. The herb is used internally to fight against most digestive problems including poor appetite, colitis, irritable bowel syndrome, constipation, and peptic ulcers.

The aim of this study was to determine the age related efficacy of ethanolic extract of A. vera gel in young, adult, old male mice. Intraperitoneal injection of alloxan monohydrate 120 mg/kg body weight was given for induction of diabetes. Ethanolic extract of A. vera gel 300 mg/kg body weight as given intraperitonealy to diabetic mice for 15 days in all age groups. Insulin was given 500µg/kg body weight. Blood glucose level, body weight, glycogen level were assessed. Our finding confirmed significant difference according to age.

Key words: Alloxan, Aloe vera, hyperglycemia, intraperitoneal, diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is one of the most challenging metabolic disorder of the 21st century that affects biochemical pathways in the body such as carbohydrates, protein and lipid metabolism. Diabetes has risen rapidly due to the factors such as urbanization, increasing obesity due to dietary changes and physical inactivity (Popkin et al., 2012). DM is particularly characterized by the excessive accumulation of free glucose in blood which is due to defects in insulin secretion, insulin action or it may be both conditions. This condition increases the risk for developing various metabolic disorders. Diabetic complication such as retinopathy, nephropathy, hypertension and neuropathy occurs in several cases. (Paneni et al., 2013; Karamanou et al., 2016). Stable glucose homeostasis is generally associated with the ability of
insulin to mediate tissue glucose uptake. Diabetes can be prevented by synthetic drugs like thiazolidinediones, sulfonylurea, meglitinide, biguanides and alpha-glucosidase inhibitors. Treatment of these drugs is costly and have several undesirable side effects such as nausea, vomiting, diarrhea, dizziness, headache, acidity, hypersensitivity abdominal upset, weakness, respiratory infections, muscle pain and indigestion (Singh et al. 2009). The incidence of diabetes increases with age (Lozzo et al., 1999). The study has shown that insulin resistance increases with age due to changes in dietary habits, increased adiposity, decreased lean muscle mass and reduced physical activity (Scheen et al. 2005).

Recent studies confirmed the insulin sensitivity improved by many herbal preparation (Ghorbani et al., 2013; Kaur and Arora, 2015). Different extracts from medicinal plant have been used traditionally to managed diabetes and these are considered as relatively in expensive, less toxic or no side effects (Gupta et al., 2008).

*Aloe vera* is a cactus like perennial plant belonging to family Liliaceae, native to North Africa. The plant has elongated pointed fleshy leaves consisting to parts outer skin and inner pulp. The plant consist of high content of phenolic compounds, glycosides (aloins), manose phosphate and glucoprotein.

Presently we investigated the effects of repeated oral administration of the extract on body weight, blood glucose level and liver glycogen level of young, adult and old male mice.

**MATERIAL METHODS**

**Preparation of Aloe vera leaf extract**

The fresh *A. vera* leaves (Voucher specimen: KJS-1) were washed thoroughly with water, peel was removed and only pulp was collected. The collected pulp was lyophilized. Extraction of lyophilized material was carried out by soxhlet method. The extract obtained was dried at 37°C in oven. The obtained yield was stored in refrigerator at 8°C until further use. The residual extract was re-suspended in distilled water and used in study as per desired concentration when needed.

**Animals**

Healthy young, adult and old male mice (*Mus musculus* Linn.) were used for present investigation. Mice were obtained from (Rajarambapu College of Pharmacy, Kasegao, Sangali). Young mice (2 month), Adult mice (4 month) and old mice (18 month) age and weighing about young mice 22 ± 2, adult mice 32 ± 2 and old mice 27 ± 2 were selected. All the animals were maintained under standard laboratory conditions, with 12 hr light and 12 hr dark cycle at temperature of 26⁰ C ± 2⁰ C in departmental animal house. The guidelines of CPCSE were followed throughout the experimentation. The animals were housed in aluminium cages having dimensions of 10” x 8” x 5” and allowed to live in groups of 3-4 animals per cage. They were fed with Amrut Mice feed, marketed from Pranav Agro Industries, Pvt. Ltd. Sangli and water *ad libitum*. The record of their body weight was maintained.

**Experimental design**

Young, adult and old male mice were divided into three groups containing 6 animals per group.

**Control group:**
Mice were given intraperitoneal injection of 0.15 M Acetate buffer pH 5.4 for 15 days.

**Diabetic group:**
Mice were given single intraperitoneal injection of alloxan 120 mg/kg body weight (Fayed et al., 1988; Helal, 2000 and Syiem et al., 2002).

**Recovery group:**
Mice from diabetic group were given intraperitoneal injection of *A. vera* leaf extract at dose of 300mg/kg body weight once a day for 15 days (Rjasekaral et al., 2004).

**Diabetic + Insulin group:**
Mice from diabetic group were given intraperitoneal injection of Insulin 500 µg/kg body weight (Rajesh Mandade, 2012).

**Determination of body weight**
Animals of both the sexes from each age group weighed before starting experiment, animals from control, diabetic and recovery group and insulin treated group. The record of these observations was maintained.

**Determination of blood glucose level**
Fasting blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. The blood glucose was determined by using a rapid glucose analyzer with a glucose strip inserted in sugar
scan digital blood glucose monitoring glucometer. The result were expressed in terms of milligram per deciliter of blood.

**Estimation of Glycogen by Anthron method (Seifer, 1950)**
The glycogen level from the liver tissue was extracted by KOH solution. This solution precipitated with ethanol and redissolved in distilled water. Thereafter, the liver glycogen content was determined by anthrone reagent and assayed by a spectrophotometer at 620 nm.

**Statistical analysis** -
The data was statistically analyzed by One way ANOVA followed by Tukey HSD test. All the values were expressed as mean ± S.E. The difference was considered significant when p<0.001.

### RESULTS AND DISCUSSION

**Table 1:** Showing body weight (gm) in control and experimental young, adult and old male mice.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Animal group (n=6)</th>
<th>Young mice</th>
<th>Adult mice</th>
<th>Old mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body weight (gm)</td>
<td>Statistical significance</td>
<td>Body weight (gm)</td>
</tr>
<tr>
<td>1.</td>
<td>Control group</td>
<td>19.4 ± 2.07</td>
<td>1:2, P&lt;0.01</td>
<td>31.2 ± 1.3</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic group</td>
<td>16.6 ± 2</td>
<td>2:3, P&lt;0.05</td>
<td>25 ± 1.58</td>
</tr>
<tr>
<td>3.</td>
<td>Recovery group</td>
<td>19.8 ± 0.83</td>
<td>2:4, P&lt;0.01</td>
<td>29 ± 1.58</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic + Insulin group</td>
<td>20.4 ± 0.83</td>
<td>3:4, P&lt;0.01</td>
<td>29.2 ± 1.3</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Number in parenthesis denote number of animals. P< 0.01 = significant, P< 0.001 = highly significant

**Table 2:** Showing blood glucose level (mg/dl) in control and experimental young, adult and old male mice.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Animal group (n=6)</th>
<th>Young mice</th>
<th>Adult mice</th>
<th>Old mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood glucose level (mg/dl)</td>
<td>Statistical significance</td>
<td>Blood glucose level (mg/dl)</td>
</tr>
<tr>
<td>1.</td>
<td>Control group</td>
<td>79.4 ± 6.22</td>
<td>1:2, P&lt;0.01</td>
<td>81.8 ± 7.52</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic group</td>
<td>229.4 ± 5.58</td>
<td>2:3, P&lt;0.05</td>
<td>227.4 ± 8.11</td>
</tr>
<tr>
<td>3.</td>
<td>Recovery group</td>
<td>125.8 ± 7.19</td>
<td>2:4, P&lt;0.01</td>
<td>130.6 ± 7.73</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic + Insulin group</td>
<td>99.2 ± 13.73</td>
<td>3:4, P&lt;0.01</td>
<td>99.6 ± 14.25</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Number in parenthesis denote number of animals. P< 0.01 = significant, P< 0.001 = highly significant
Table 3: Showing liver glycogen level (mg /100 mg tissue) in control and experimental young, adult and old male mice.

<table>
<thead>
<tr>
<th>Sr. N o.</th>
<th>Animal group (n=6)</th>
<th>Young mice</th>
<th>Adult mice</th>
<th>Old mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycogen level mg/100mg tissue</td>
<td>Statistical significance</td>
<td>Glycogen level mg/100mg tissue</td>
<td>Statistical significance</td>
</tr>
<tr>
<td>1.</td>
<td>Control group</td>
<td>5.92 ±0.35</td>
<td>1:2, P&lt;0.01</td>
<td>11.99 ±0.41</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetic group</td>
<td>4.04 ±0.26</td>
<td>2:3, P&lt;0.01</td>
<td>6.58 ±0.59</td>
</tr>
<tr>
<td>3.</td>
<td>Recovery group</td>
<td>5.35 ±0.21</td>
<td>2:4, P&lt;0.01</td>
<td>10.58 ±0.61</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetic+Insulin group</td>
<td>5.83 ±0.43</td>
<td>3:4, P&lt;0.01</td>
<td>10.88 ±0.24</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Number in parenthesis denote number of animals. P< 0.01 = significant, P< 0.001 = highly significant

This study was designed to investigate the age related hypoglycemic effect of ethanolic extract of A. vera in young, adult and old male mice. This animal model is known to mimic pathophysiological aspects of type I and type II diabetes in human patients (Lozzo et al., 1999; Cowie et al., 2006; Eddouks et al., 2012). There are various mechanisms that have been associated with antihyperglycemic activities of medicinal plants which are peripheral utilization of glucose, inhibition of carbohydrate metabolizing enzymes, increased synthesis of hepatic glycogen, stimulation of pancreatic insulin release and inhibition of hepatic glucose production (Xu Z et al., 2008; Krishnasamy et al., 2016). The reduction of fasting blood glucose level by the extract may have exerted this effect through one or more of these mechanism (Defronzo RA and Goodman AM, 1995). Postprandial glucose clearance by the liver translates to glycogen synthesis and storage which may be due to enhanced insulin release from β cells (Shalev A. 1999). Body weight is a sensitive indicator that reflects the state of health of experimental animals. In the present study treatment on diabetic mice showed decrease in body weight loss, which indicates the prevention of muscle tissue damage and protein loss that occur in hyperglycemic condition. The extract protected the weight loss induced by alloxan shows correlation with the earlier studies from other plant extracts (Badole et al., 2006). DM impairs the normal capacity of the liver to synthesise glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis. Decrease in hepatic glycogen was observed in this study. Treatment with A. vera significantly increased liver glycogen in all age group mice. The effective results might be due the precence of aloins in this ethanolic extract of A. vera responsible for the hypoglycemic activity. The alkaloids promotes the regeneration of β cells, hence it restore the secretion of insulin (Piero et al., 2015). In present study diabetic mice from all age group shows effective hypoglycemic activity of A. vera. Beta cell proliferation decreases with age, but extract of A. vera shows capacity of regeneration in beta cells in young, adult and old age group mice. This study found that there was significant decrease in the glucose level in all age groups.

REFERENCES


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