Anti-diabetic and Anti-obese activity of Ethanolic Extracts of Polyherbal Drug (Allium sativum, Mangifera indica and Vinca rosea) in Streptozotocin induced Diabetic Rats

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Abstract

The ethanolic extract of Allium sativum, Mangifera indica and Vinca rosea formulations were experimented for its anti-diabetic and anti-obese properties in Streptozotocin induced diabetic male Wistar rats. The ethanolic extract of 50mg/kg, p.o. as a single dose used as treatment to the diabetic rats for a period of 24 days. The body weight was assessed for a time interval of 4 weeks. The body weight was found to be improved in the treatment group with an increase in changes compared to control and STZ induced diabetic group. This result proved the efficiency of plant extracts formulation as an anti-obese drug. The experimental rats subjected to OGTT showed decreased glucose levels in the plant extract treated group compared to the control and diabetic rats. From the above results, it is concluded that the plant extract formulation possesses significant anti-diabetic and anti-adipogenicity effects in STZ-induced diabetic rats.

Keywords - Allium sativum, Mangifera indica, Vinca rosea, Anti-obese, Polyherbal therapy, Anti-diabetic.

INTRODUCTION

Diabetes mellitus is a severe chronic disorder arising from a diverse group of chronic diseases characterized by decreased glucose, meat, and protein metabolism resulting from the plurality of environmental factors interacting together (Ramachandran et al., 2011). This has a major impact on patient’s health, standard of living and sustainability as well as on the medical system's economy. Obesity is one of the major effects of diabetes where the insulin resistance is developed along with the β-cell dysfunction. The various Indian herbs showed powerful suppression of this disease, particularly the effect of polyherbal therapy is adversary as well as synergistic due to their combined effects (Mardi et al., 2010). Allium sativum is an Indian herb that has a strong effect as an anti-adipogenic agent. Mangifera indica stimulates the antagonistic action towards the elevation of blood glucose levels while Vinca rosea is both anti diabetic and anti-cancerous herb. In a combinational therapy, the presence of one compound in
a plant elicits its action that maybe deficit in another thus saturating the necessary benefits (Grover et al., 2002). The efficiency of action is higher compared to single herbal actions.

**MATERIALS AND METHOD**

**Preparation of Plant Extracts**

Leaves of *Mangifera indica*, *Vinca rosea* and bulbs of *Allium sativum* are collected from the location of Vadavalli area in Coimbatore district. The leaves, bulbs were washed and shade dried for 10-14 days. The leaves and bulbs were pulverized separately to coarse powder (5g) after dried and homogenized with ethanol (50ml each). The powders were solubilized and days continually. The prepared plant-solvent mixture was filtered with Whatman filter paper (no.#1). The obtained solvent was placed in petri-plates in hot air oven (50ºC-80 ºC) and allowed for drying. The extracts were mixed in the ratio 1:1:1 for future use using ethanol.

**Animals**

*Experimental Design* - Adult male albino Wistar rats (6 weeks), weighing 150 to 200g were used for the present anti-diabetic study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature-controlled animal house with a constant 12h light/dark schedule. The animals were fed with standard rat pelleted diet and clean drinking water was made available *ad libitum*. All animal procedures were performed after approval from the ethical committee and in accordance with the recommendations for the proper care and use of laboratory animals.

*Reagents* - Streptozotocin (500 mg, S-0130, Sigma-Aldrich), Nicotinamide (100 g, N-3376, Sigma-Aldrich), Sodium Citrate (Mw: 294.10), Citric Acid (Mw: 210.10), Sucrose 10%, Distillate water, Sodium chloride (NaCl 0.9%).

*Induction of Diabetes Mellitus* - The animal divided into 4 groups of six animals each. The animals are kept overnight fasting and check the initial fasting blood glucose from tip of rat tail vein. Streptozotocin was dissolved in citrate buffer (pH 4.5) and Nicotinamide was dissolved in normal saline. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 min after the i.p administration of 120 mg/kg of nicotinamide. Hyperglycaemia was confirmed by the elevated levels of blood glucose were determined at 72 h. The animals with blood glucose concentration more than 250mg/dl will be used for the study.

**RESULTS**

The results in table 1 shows the significant decrease in the body weight of diabetic rats treated with the plant extract formulation for 4 consecutive weeks. Diabetes induced rats showed substantial loss in the body weight compared to control group, while there was a significant increase in the treatment group.

Table 1. Effect of Plant extracts on Body weight of STZ induced diabetic rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Control</th>
<th>Only STZ</th>
<th>STZ + STD</th>
<th>STZ + PLANT EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>127.6±</td>
<td>130.8±</td>
<td>134±</td>
<td>138.6±</td>
</tr>
<tr>
<td></td>
<td>2.315</td>
<td>6.53</td>
<td>4.796</td>
<td>3.234</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Week 2</th>
<th>178±5 727</th>
<th>125.6±31.95</th>
<th>167.2±7.716</th>
<th>132.2±33.18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 3</td>
<td>182.2±3.527</td>
<td>86.8±35.61</td>
<td>142.2±36.62</td>
<td>145.8±36.53</td>
</tr>
<tr>
<td>Week 4</td>
<td>178.8±7.158</td>
<td>60±36.83</td>
<td>158.6±40.77**</td>
<td>119±49.0**</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one-way ANOVA followed by Dunnett’s; **P< 0.05 calculated by comparing treated group with control group.

Fig 1. Changes in body weight of STZ induced diabetic animals’ animals at different time interval

The body weight was elevated in normal rats, while the diabetic rats showed significant decrease. After a treatment of 21 days with the plant extract formulation, the rats showed reduction in their body weight. STZ diabetic induction caused gain in body weight which was upturned by the action of plant extract formulations.

**Oral Glucose Tolerance Test**

Glucose (2 g/kg) was fed 30 min after pre-treatment with distilled water, HALEC and glibenclamide. Blood glucose levels were measured every 60 minutes after glucose load to access the effect of extract on blood glucose levels of the glucose loaded animals. The blood glucose was measured using blood glucose test strips and glucometer (Kesari et al., 2006). For the oral glucose tolerance test, the experimental animals were grouped into 4 as follows:

Table 2. Grouping of animals for OGTT
Table 3. Effect of Plant Extracts on OGTT in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CONTROL</th>
<th>GLUCOSE</th>
<th>GLUCOSE + Glibenclamide (2 mg/kg p.o)</th>
<th>GLUCOSE + Plant Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Distilled water</td>
<td>58.5±1.854</td>
<td>54.1±0.856</td>
<td>54.5±2.901</td>
</tr>
<tr>
<td>Group II</td>
<td>Only Glucose (2 g/kg p.o)</td>
<td>150.75±0.8539</td>
<td>54.5±2.901</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>Glibenclamide (2 mg/kg p.o) + Glucose (2 g/kg p.o)</td>
<td>78.25±0.8539</td>
<td>234.3±2.955***</td>
<td>180.3±1.55***</td>
</tr>
<tr>
<td>Group IV</td>
<td>Extract formulation + Glucose (2 g/kg p.o)</td>
<td>79±2.041</td>
<td>189±0.4082****</td>
<td>180.3±1.55***</td>
</tr>
</tbody>
</table>

Each value represents Mean ± SEM. One-way ANOVA, followed by Dunnett comparison was performed. (ns – non significant, ***P<0.001, **P<0.01, *P<0.05).

Table 3 represents the OGTT in glucose-loaded rats which were observed for every 1 hour with maximum efficacy at the 5th hour. The glucose tolerance capacity was compared between control group and other groups respectively. The level of glucose was found to be increased (peak) at the 3rd hour and gradually decreased on 4th and 5th hour. Treatment group showed significant decrease compared to the control and vehicle-treated group while there was an insignificant difference between the normal and treatment group.

DISCUSSION

The body weight enhanced with the treatment of plant extract formulations, along with the duration of time. The body weight was found to be directly comparative with the time period. Results of the present study are supported by the findings of Saleem et al., (2019) where it was observed with a significant decrease in the body weight due to the presence of various phytocompounds. As the action of lipid regulatory systems, the body weight was also gained in the later weeks, this revelation was concluded in the findings of Barik et al., (2014). The effect of plant extract formulation on the oral glucose tolerance test was observed from table 2 where the experiment maybe suggestive for the treatment of hyperinsulinemia along with the improvement of body weight regulation. The reports from the work of Vivek et al., (2016) showed prevention of blood glucose level with diabetic inhibition. Zhaoxia Liu et al., (2013) reported that compared to diabetic group, DTH extract treated group showed significant hypoglycaemic effect and steady decline which exhibited a similar effect to metformin on OGTT. The fall in the blood glucose level are due to insulinogenic activity of the plant extracts Ren et al., (2008). This demonstrates the hypoglycemic property of the plant extract formulations thereby proving its anti-diabetic activity.
CONCLUSION

The body weight of the animals showed improvement with the dosage of plant extract formulations prepared, thereby demonstrating the anti-obese property. *Allium sativum* is a powerful fat burner which along with *Mangifera indica* and *Vinca rosea* regulates a synergistic effect for treating diabetes. The oral glucose tolerance test exhibited a fall in the levels of glucose, which shows the antihyperglycemic and antiadipogenic property of the herbal drug formulation.

Conflict of Interest

The authors report no declarations of conflict of interests.

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