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Original Research Article

Evaluation of Antidiabetic Activity of Chromolaena Odorata Leaves Extract In Streptozotocin-Induced Rats

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Abstract

The burden of diabetes on public health is multifaceted, ranging from consequences to the shortcomings of available treatments. It is necessary to develop a novel diabetes treatment made of plants that has fewer or no side effects. In several nations, Chromolaena odorata is widely used as a conventional antidiabetic medication. The aim of this investigation was to assess the antidiabetic potential of Chromolaena odorata leaf extract in rats that had been given diabetes. Rattusnovergicus was divided into six groups: normal control, diabetic control, glibenclamide treatment group, and three doses of Chromolaena odorata leaf extracts (100 mg/kg & 200 mg/kg BW). For a period of five weeks, an oral dose of Chromolaena odorata extract was administered daily. There was a comparison between the group's animal body weight, blood glucose, insulin, and pathological alterations in the pancreas. The paired t-test or one-way ANOVA were used to analyse the data. According to our findings, there was a substantial ($p<0.05$) difference in body weight between the diabetes group and all Chromolaena odorata treatment groups. When comparing the animals in the glibenclamide and Chromolaena odorata group to the diabetic control group, there was a noticeable drop in blood glucose levels. When comparing the insulin levels of Chromolaena odorata groups of animals to those of diabetic control animals, a noteworthy elevation was also observed. Animals given Chromolaena odorata extract showed signs of regeneration in their beta cells. The ethanol extract of Chromolaena odorata leaves may have antidiabetic properties, according to the study's result.

Keywords: Diabetes, Chromolaena odorata, Glibenclamide.

Introduction

Diabetes is a long-term metabolic disease marked by elevated blood glucose levels, or hyperglycemia. An inevitable consequence of unchecked diabetes is hyperglycemia, which can cause permanent harm and malfunctions in the heart, blood vessels, kidneys, eyes, and nerves [1]. Diabetes has a substantial impact on the quantity of both microvascular and macrovascular diabetic complications. The macrovascular complications of diabetes include peripheral vascular diseases, heart attacks, and strokes; the microvascular

complications include retinopathy, neuropathy, and nephropathy. Both issues have the potential to raise mortality and lower quality of life [2]. Oral hypoglycemic agents (OHA's) are the most often prescribed medication for the treatment of diabetes; yet, a number of side effects have been documented [3,4]. There is increasing interest in herbal medicine as an antidiabetic medication made of plant-derived components to lessen side effects linked to OHAs5. Numerous therapeutic herbs are employed as antidiabetic medicines, pharmacological testing has demonstrated the effectiveness of several of these plants. [6].

People who live distant from official health care facilities, especially those with little financial resources, can benefit from medicinal plants. The World Health Organisation (WHO) is a major proponent of drug discovery and development research, particularly with regard to medicinal plants that have been used for centuries and have been shown to be effective in the treatment and control of various diseases [7]. Traditional diabetic treatment has been derived from more than a thousand plant species [8]. The member of the Compositae family, Chromolaena odorata L.

One of the most effective plant families with hypoglycemic action is Asteraceae. Although this plant is native to South and Central America, it has now spread around the world, particularly to tropical and subtropical regions. Because of its pharmacological qualities, including its anti-microbial activities [9,10], antioxidant activities [11,12], and anti-inflammatory activities [13], C. odorata has been utilised traditionally. There have also been reports of anti-diabetic properties from Chromolaena odorata leaves [14-18]. Nevertheless, the effectiveness of Chromolaena odorata leaf extract in treating diabetic-related illnesses has only been assessed in a few number of research studies to date. This study was designed to assess the improvement of variables related to diabetes, such as body weight, blood glucose level, blood insulin level, and pancreas, in streptozotocin-induced diabetic rats after ethanol leaves extract of Chromolaena odorata was administered.

Materials and Methods

Study design, environment, and experimental animals are male Wistar rat in good health, weighed. They weighed 180-200 grams and were 10–12 weeks old when they were bought from the Animal Breeding House Unit. The animals were housed at room temperature, between 22 and 25 degrees Celsius in Animal House. The animals were kept in an environment with 12 hours of light and 12 hours of darkness. They also had unlimited access to water and regular rat pellets.

Six groups of animals were used: Control groups, diabetic without treatment groups, and diabetes with standard glibenclamide treatment & Chromolaena odorata extract groups with doses of 100 & 200 mg/kg body weight (BW), designated as Group Co100 & Group Co200 respectively and glibenclamide treatment (Group glibenclamide). There were six animals assigned to each group.

On the day of diabetic induction the body weight, insulin level, and fasting glucose level were assessed after the animals had been acclimated to the laboratory conditions for a week. Then, using overnight fasted rats, a single intraperitoneal injection of 45 mg/kg BW Streptozotocin dissolved in cold citrate-buffered saline (0.1 mol/L; pH 4.5) was used to induce diabetes in all groups (except from Group control). Group control was given saline that had been citrate-buffered [19]. The animals in each group had their body weight, insulin level, and fasting glucose level remeasured on day three following induction. Glibenclamide and Chromolaena odorata extract were began to be administered. The mice in Gdiabetes did not receive any further medication, while Group glibenclamide animals received 0.45 mg/kg BW glibenclamide. For 35 days, oral leaf extract

was administered to the Chromolaena odorata groups at doses of 100 & 200mg/kg BW respectively. The body weight and fasting glucose level were measured every week until week 5 (denoted as T3, T4, T5, and T6) while the level of insulin was re-measured on T6 only.

Preparation of Chromolaena odorata leaves extract: 1 kilograms of fresh Chromolaena. odorata leaves were dried for two weeks at room temperature, grounded using a grinder mill, and the material were macerated with 80% ethanol using the Soxhlet extractor apparatus for 18 hours. The liquid extract was then filtered using Whatman filter paper. The residue was remacerated twice with fresh solvent, every day. All the filtrates were concentrated using a vacuum rotary evaporator and dried in a hot air oven at 180C. The final extract obtained from Chromolaena odorata dried leaves was 100g which then stored in a wide-mouthed and tightly closed bottle at 4°C until used.

Measurement of body weight: The body weight in all experimental animals was firstly measured before the rats were induced with streptozotocin which was performed after the seventh day of acclimatization (T1). The animals were then weighed every week until the end of observation week (T6).

Measurement glucose level: Blood (±1 mL) was drawn from overnight fasted rats at the caudal vein of the rat's tail, then frozen and centrifuged to separate the serum. The serum was then used to measure the glucose level. Rats were considered diabetics if the fasting blood glucose exceeded 200 mg/dL [18]. Glucose level was measured using the One touch Glucometer.

Statistical Analysis: Paired t-test was used to analyze the difference of mean insulin level between T1 and T6 in each group. One-way analysis of variance (ANOVA) with post hoc test using Least Significant Difference (LSD) test was employed to compare the body weight, and glucose level between and within-group at different times point. The results were considered to be significant at $p<0.05$.

Ethics approval: After having approval from Animal ethics committee from Shadan Medical College, Hyderabad. The studies was further proceed & has reviewed this experimental protocol with ethical approval issued on 10 May 2014.

Results

Streptozotocin-induced diabetes: The successful diabetic induction was marked by a sudden increase in blood glucose level (>200 mg/dL) after induction in the diabetic groups (Group diabetes, Group gliben, Group Co100 & Group Co200). No visible signs of toxicity such as excitement, restlessness, respiratory distress, convulsions, or coma were observed. All animals remained alive during 35 days of observation.

The effect of Chromolaena odorata on body weight: The body weight of experimental animals was increased in all groups, except animals within Group diabetes in which continued to decline each week. A significant difference in body weight was observed among groups for each time point from T3 to T6 ($p<0.001$). The body weight of animals within Chromolaena odorata groups (GCo100 & GCo200) had similar response trend to Group glibenclamide and animals within Group Chromolaena odorata 200 had the greatest increase. Rats within Group Chromolaena odorata 200 had heavier body weight at all-time points (T2- T6) than Group gliben although not statistically significant. Posthoc analysis indicated that body weight of animals of Gdiabetes was significantly different compared to each Chromolaena odorata group (Group Co100 & Group Co200) in T3-T6, ($p<0.05$).

The effect of Chromolaena odorata extract on glucose level: A sharp increase in glucose level was observed in diabetic induced animals after Streptozotocin injection. In

Group diabetes animals, the glucose level remained high over the study period In contrast, the glucose level of rats within Chromolaena odorata groups (GCo100 & GCo200) and the glibenclamide group (Group gliben) decreased gradually. The glucose levels were significantly different among groups in T3-T6 with $p<0.001$. Glucose level of animals within Group diabetes group was significantly different compared to each Chromolaena odorata group (GCo100 & GCo200 in T3 to T6 ($p<0.001$ for each time point). The level of glucose within GCo200 had the sharpest decline compared to the other Chromolaena odorata groups with lower doses.

The effect of Chromolaena odorata on blood insulin levels. The level of insulin was measured before diabetic induction (T1) and at the end of observation at week 5 (T6). The insulin level of animals within Gcontrol and Gdiabetes in T6 was lower significantly compared to T1, with $p<0.05$. In contrast, the levels of insulin in both Chromolaena odorata groups and Group gliben had an increase trend.

Insulin level of rats within all Chromolaena odorata groups increased significantly with Group Chromolaena odorata 200 had the highest increase. There was a significant difference in insulin level between the Group diabetes compared to each of Chromolaena odorata treatment groups (Co100 & Co200) with $p<0.001$.

Table 1: The levels of blood insulin (pg/mL) before and after treatment with Chromolaena odorata

Groups	Before diabetic induction (mean ±SD)	After 5 weeks of treatment (mean ±SD)
Control	255.47±2.00	251.05±2.80
Diabetes	122.37±1.66	119.48±2.33
Glibenclamide	120.31±2.90	248.25±2.77
Co 100	125.05±5.00	250.12±5.64
Co 200	120.08±9.08	249.31±3.72

Group Control: Normal rat, Group Diabetes: Diabetic rat, Group glibenclamide: Diabetic rat with glibenclamide treatment, Group Co100: Diabetic rat and treatment with Chromolaena odorata extract 100mg/kg BW & Group Chromolaena odorata 200: Diabetic rat and treatment with Chromolaena odorata extract 200mg/kg BW.

Discussion

In this study, diabetic was induced by intraperitoneal injection of streptozotocin that contributes to the functional defects of the pancreatic beta cells [20,21], resulting in limited endogenous insulin production, hyperglycemia, and weight loss. It also affects the glucose level by destroying the insulin produced by beta cells in the islet of Langerhans [22,23]. Assessment of the glucose level after induction ensured that the animals were in the diabetic state by which the glucose level was above 200 mg/dL in all animals within Group diabetes, Group glibenclamide, Group Chromolaena odorata 100 & Group Chromolaena odorata 200. This diabetic induction induced the reduction of body weight as the result of insulin deficiency that prevents the utilization of glucose for an energy source and induces the consumption of stored fats and muscle protein for energy. Our data suggested that the administration of Chromolaena odorata leaves extract increased the body weight significantly in a dose dependent manner. Rats within GCo200 had the most increase in body weight compared to the lower doses (i.e. GCo100). This result is

similar to another study that used the higher dose (400 mg/kg BW) [5]. The increase in body weight was similar to animals that were treated with glibenclamide, suggesting that Chromolaena odorata extract could prevent the muscle wasting due to hyperglycemic status and improved the metabolic activity [20] Our data also suggested that Chromolaena odorata extract could reduce the level of blood glucose. The greatest reduction in blood sugar levels was observed in those that treated with the highest dose of Chromolaena odorata extract and this was similar in the group treated with glibenclamide. Previous studies have reported the hypoglycemic properties of Chromolaena odorata extract [24].

Leaves of Chromolaena odorata have been reported to have a lowering effect on blood glucose that may be attributed to the presence of phenols, flavonoids, alkaloids, tannins, and saponins [25,26] The presence of these phytochemicals stimulates the production of insulin in the islet of Langerhans leading to a reduction in blood glucose level. Increase of insulin secretion, inhibition of internal glucose production, inhibition of gut glucose absorption, and regeneration Increase of insulin secretion, inhibition of internal glucose production, inhibition of gut glucose absorption, and regeneration of beta cells may explain the hypoglycemic activity of Chromolaena odorata as these mechanisms have been reported for lowering glucose level [27-30].

However, further studies are needed to elucidate the most prominent mechanism. Nevertheless, one of the mechanisms is to increase insulin secretion and to improve the regeneration of pancreatic beta cells. One of diabetic characteristics is impaired insulin secretion from beta cells and insulin recognition in the tissues. The decrease in insulin release due to beta cells dysfunction will lead to insufficient maintenance of normal glucose levels [31]. Our study indicated that the level of insulin increased in all Chromolaena odorata treated groups compared to those untreated animals, the highest rise was observed within group treated with the highest dose. A previous study also suggested that Chromolaena odorata extract affected the extent of restoration of beta cell function [32] and Chromolaena odorata extract could regenerate the beta cells to almost similar to the normal control group. Further studies are required to elucidate the mechanism. There are some limitations of this study. This study used a relatively small number of animals for each group. Nevertheless, the measurements in this study were carried out in triplicate. This study assessed three indicators only to evaluate improvement state; therefore, further assessment to include more indicators to evaluate the diabetic state will provide valuable information.

Conclusion:

This study demonstrated the antidiabetic activities of Chromolaena odorata leaves extract in streptozotocin-induced diabetic rats. The administration of this extract for five weeks suggested the improvement of diabetic-associated conditions: increased the body weight, reduced the blood glucose level, restored the blood insulin level, and increased the insulin expression in the pancreatic beta cells. These results provide basic information for further study to isolate and characterize the active compounds and to evaluate their action mechanism.

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