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Pharmacological Evaluation of Antidiabetic Activity of *Chromolaena Odorata* Leaves Extract in Streptozotocin-Induced Rats

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ABSTRACT

Diabetes poses a public health burden range from the inadequacy of current therapies to complications. A new diabetic therapy derived from plants with less or no adverse effects is needed. *Chromolaena odorata* is commonly used as a traditional antidiabetic agent in some countries. The objective of this study was to evaluate the antidiabetic activity of *C. odorata* leaves extract in diabetic-induced rats. Six groups of *Rattus novergicus* were used: three concentrations of *C. odorata* leave extracts (100mg/kg body weight (BW), 200mg/kg BW, and 400mg/kg BW), normal control, diabetic control, and glibenclamide treatment group. *C. odorata* extract was administrated orally each day for five weeks. The animal body weight, blood glucose level, insulin level, and pathological changes of the pancreas were compared among groups. Data were analyzed using paired t-test or one-way ANOVA with post hoc analysis as appropriate. Our data indicated a significant difference in body weight between the diabetic group and all *C. odorata* treatment groups ($p<0.05$). There was a significant decline in blood glucose level in animals within *C. odorata* and glibenclamide group compared to diabetic control group. A significant increase in insulin level was also seen in animals within *C. odorata* groups compared to diabetic control animals. Beta cells regeneration was observed in animals treated with *C. odorata* extract. In conclusion, this study suggested that the ethanol extract of *C. odorata* leaves has antidiabetic activity. A study is warranted to identify the compound for further investigations.

Keywords: Chromolaena odorata; diabetes; hyperglycemia; streptozotocin

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INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by hyperglycemia, a high level of glucose in the blood. Hyperglycemia is a common effect of uncontrolled diabetes and it could lead to long-term damage and functional disorders of various tissues and organs such as eyes, kidneys, nerves, heart, and blood vessels[1]. Diabetes significantly affects the number of microvascular and macrovascular diabetic complications[2]. Heart attack, stroke, and peripheral vascular diseases are the macrovascular complication of diabetes, while retinopathy, neuropathy, and nephropathy are the microvascular complication of the disease[3]. Both complications could reduce the quality of life and increase mortality[4].

The most common drug used in treating diabetes is oral hypoglycemic agents (OHAs); however, several adverse effects have been reported[5, 6]. Therefore, finding a new antidiabetic drug from plant-derived compounds is a growing interest in herbal medicine to reduce OHAs-associated adverse events[7, 8]. Several medicinal plants are used as antidiabetic agents, and some of them have shown their efficacy through pharmacological evaluation[9]. Medicinal plants are beneficial for people who are far from formal health service centers, including those with low economic capacity. The World Health Organization (WHO) strongly supports researches related to the discovery and development of new drugs, especially the medicinal plants that have been traditionally used by the community and proven to be able to control and cure the diseases[10].

More than 1000 plants species have been used as traditional medicine for diabetes[11]. *Chromolaena odorata* L (*C. odorata*), which belongs to the family Compositae or Asteraceae, is one of the most potent plants that have hypoglycemic activity. This plant originates from South and Central America but now has spread over the world especially in the tropics and subtropics areas. *C. odorata* has been used traditionally for its pharmacological properties such as anti-microbial activities[12, 13], antioxidant[14, 15], and anti-inflammatory[16]. The leaves of *C. odorata* also has been reported to have anti-diabetic activity[17-20]. However, to date, only a limited number of studies have evaluated the improvement of diabetic-associated conditions after *C. odorata* leaves extract administration. This study was set up to evaluate the improvement of diabetic-associated conditions including body weight, blood glucose level, blood insulin level, and the pancreas after the administration of ethanol leaves extract of *C. odorata* in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Experimental animals, study design and setting

Male healthy Wistar rats (*Rattus novergicus*), weighing 170-190 grams and age of 10-12 weeks, were purchased from Animal Breeding House Unit, University of Gadjah Mada, Yogyakarta, Indonesia. The animals were kept in polypropylene cages in the Animal House of Center for Food and Nutrition Studies at room temperature 22-25^{o}C. The animals were maintained under 12 hours light and 12

hours dark and allowed free access to standard rat pellets and water *ad libitum*.

Animals were divided into six groups: three control groups (non-diabetic control group ($G_{control}$), diabetic without treatment group ($G_{diabetes}$), and diabetic with standard glibenclamide treatment (G_{gliben})) and three *C. odorata* extract groups with a dose of 100, 200, and 400 mg/kg body weight (BW), assigned as G_{Co100}, G_{Co200}, and G_{Co400}, respectively. Six animals were allocated for each group. After the animals were acclimatized to the laboratory conditions for one week, the body weight, fasting glucose level, and insulin level were measured on the day of diabetic induction (assigned as time 1, T1). Diabetic was then induced in all groups, excluding $G_{control}$, by a single intraperitoneal injection of 45 mg/kg BW streptozotocin (Nacalai Tesque, Kyoto, Japan) dissolved in cold citrate-buffered saline (0.1 mol/L; pH 4.5) on overnight fasted rats[21]. $G_{control}$ received cold citrate-buffered saline[22, 23]. On day 3 after induction (assigned as time 2, T2), the body weight, fasting glucose level, and insulin level of the animals within all groups were remeasured. On T2, administration of *C. odorata* extract and glibenclamide was started. G_{gliben} animals received 0.45 mg/kg BW glibenclamide and animal within $G_{diabetes}$ did not receive any other treatment. *C. odorata* groups (G_{Co100}, G_{Co200}, and G_{Co400}) received oral leaves extract with a dose of 100, 200, and 400 mg/kg BW, respectively; daily for 35 days. 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. Upon completion of the experiment, the animals were euthanized by injecting ketamine followed by decapitating[24].

Preparation of *C. odorata* leaves extract

Twenty-five kilograms of fresh *C. odorata* leaves were dried for two weeks at room temperature, grounded using a grinder mill, and seventeen kilograms of the material were macerated with 80% ethanol using the extractor apparatus for 24 hours. The liquid extract was then filtered using Whatman filter paper. The residue was re-macerated twice with fresh solvent, every 24 hours. All the filtrates were concentrated using a vacuum rotary evaporator and dried in a hot air oven at 40^0C. The final extract obtained from seventeen kilograms of *Chromolaena odorata* dried leaves was 150g 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

Venous 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 GOD-PAP enzymatic colorimetric test using Glucose GO FS 10 kit (Diagnosis Systems, Holzheim, Germany) in triplicate as per manufacturer's recommendation. The absorbance was read using UV-1800 UV-Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 500 nm wavelength [25]. The absorbance value was then changed into blood glucose level (mg/dL) as instructed.

Measurement of insulin level

The level of insulin was measured at T1 and at the end of the study (T6). The method is based on the direct sandwich technique of two insulin monoclonal antibodies that reacts with the blood serum using rat insulin ELISA kit (RayBiotech, Georgia, US). The absorbance was immediately read at 450 nm wavelength and the absorbance value was converted into blood insulin level (pg/mL).

Immunohistochemical study

To examine any changes in pancreatic beta-cells (β cells) after treatment, the pancreas was harvested from the euthanized rats and was stained using a mouse anti-insulin primary antibody (Abcam, Cambridge, UK). The tissue was then incubated with biotinylated universal secondary antibody (Novocastra Lab Ltd., Newcastle, UK), streptavidin-peroxidase, and stained with substrate solution 3'-diaminobenzidine (DAB). Sections were counterstained with Dako Harris Hematoxylin (Agilent, California, US) as instructed in manufacturers' protocol. Insulin expression was observed using a light microscope. Cells with positive insulin expression appear in dark brown granules contained in the cytoplasm of beta-cells, while cells with negative protein expression appear in blue/violet color.

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 $p<0.05$. SPSS ver. 24 software was used for data processing and analysis.

Ethics approval

The ethics committee for Animal Care of the General Hospital Surakarta Indonesia has reviewed this experimental protocol and passed the ethics feasibility test with ethical approval number 614/V/HREC/2019, issued on 10 May 2019.

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 ($G_{diabetes}$, G_{gliben}, G_{Co100}, G_{Co200}, and G_{Co400}) (**Table 1**). 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.

Table 1. The blood glucose levels (mg/dL) of rats before and after induction with streptozotocin 45 mg/kg body weight

Group of animals	Before Induction (mean ± SD)	After Induction (mean ± SD)
$G_{control}$	68.03 ± 1.81	69.39 ± 1.43
$G_{diabetes}$	68.88 ± 2.52	274.70 ± 7.98

G_{gliben}	71.26 ± 6.98	261.90 ± 7.40
G_{Co100}	68.88 ± 1.39	267.87 ± 6.08
G_{Co200}	67.12 ± 1.40	266.85 ± 6.09
G_{Co400}	68.20 ± 3.33	271.14 ± 4.49

$G_{control}$: normal rat, $G_{diabetes}$: diabetic rat, G_{gliben}: diabetic rat with glibenclamide treatment, G_{Co100}: diabetic rat and treatment with *Chromolaena odorata* extract 100mg/kg BW, G_{Co200}: diabetic rat and treatment with *C. odorata* extract 200mg/kg BW, G_{Co400}: diabetic rat and treatment with *C. odorata* extract 400mg/kg BW.

The effect of *C. odorata* on body weight

The body weight of experimental animals was increased in all groups, except animals within $G_{diabetes}$ in which continued to decline each week (**Fig.1**). 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 *C. odorata* groups (G_{Co100}, G_{Co200}, and G_{Co400}) had similar response trend to G_{gliben} and animals within G_{Co400} had the greatest increase. Rats within G_{Co200} and G_{Co400} had heavier body weight at all time points (T2-T6) than G_{gliben} although not statistically significant. Post-hoc analysis indicated that body weight of animals of $G_{diabetes}$ was significantly different compared to each *C. odorata* group (G_{Co100}, G_{Co200}, and G_{Co400}) in T3-T6, ($p<0.05$).

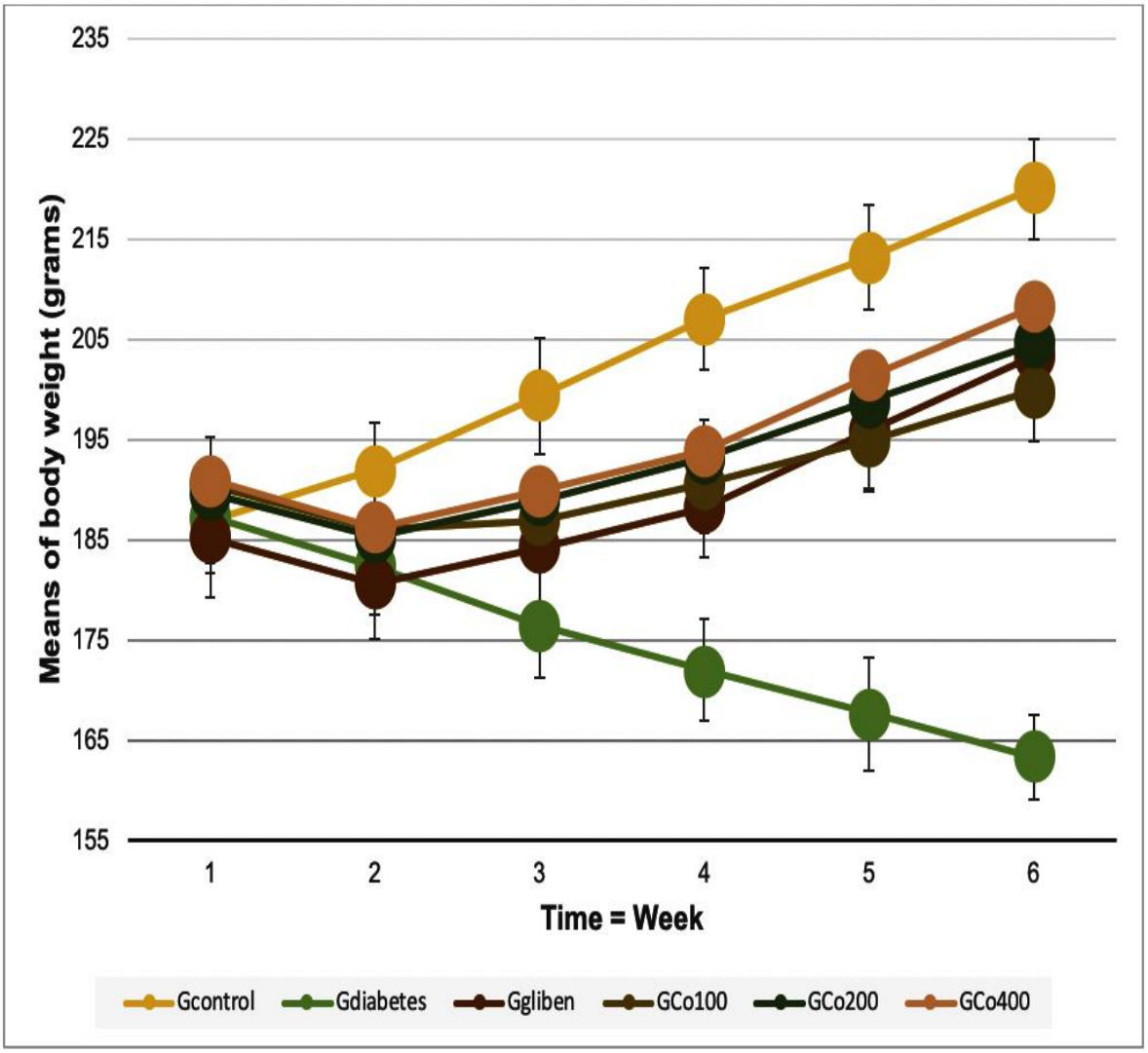

Figure 1. The average of body weight over time between groups after administering of *Chromolaena odorata* leaves extract

The effect of *C. odorata* extract on glucose level

A sharp increase in glucose level was observed in diabetic-induced animals after streptozotocin injection. In $G_{diabetes}$ animals, the glucose level remained high over the study period (**Fig.2**). In contrast, the glucose level of rats within *C. odorata* groups (G_{Co100}, G_{Co200}, and G_{Co400}) and the glibenclamide group (G_{gliben}) decreased gradually. The glucose levels were significantly different among groups in T3-T6 with $p<0.001$. Glucose level of animals within $G_{diabetes}$ group was significantly different compared to each *C. odorata* group (G_{Co100}, G_{Co200}, and G_{Co400}) in T3 to T6 ($p<0.001$ for each time point). The level of glucose within G_{Co400} had the sharpest decline compared to the other *C. odorata* groups with lower doses.

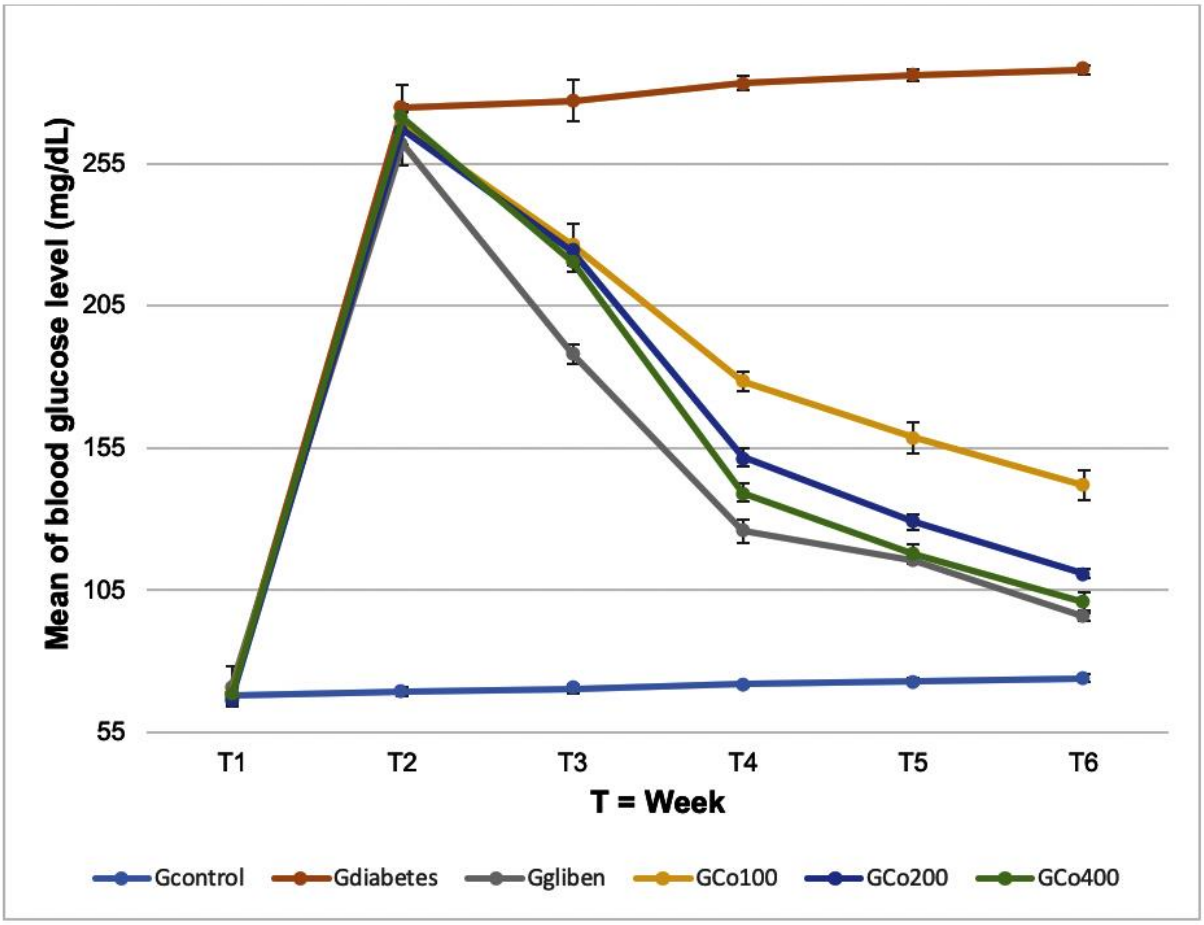

Figure 2. The average of glucose level over time between groups after administering of *Chromolaena odorata* leaves extract

The effect of *C. 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 $G_{control}$ and $G_{diabetes}$ in T6 was lower significantly compared to T1, with $p<0.05$ (**Table 2**). In contrast, the levels of insulin in both *C. odorata* groups and G_{gliben} had an increase trend. Insulin level of rats within all *C. odorata* groups increased significantly with G_{Co400} had the highest increase. There was a significant difference in insulin level between the $G_{diabetes}$ compared to each of *C. odorata* treatment groups (G_{Co100}, G_{Co200}, and G_{Co400}), with $p<0.001$.

Table 2. 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)	p-value
$G_{control}$	585.74±5.00	582.02±6.80	0.021
$G_{diabetes}$	421.37±2.66	417.48±3.33	0.024
G_{gliben}	420.31±3.90	545.93±4.73	<0.001
G_{Co100}	422.08±4.70	442.25±3.97	0.001
G_{Co200}	420.31±5.00	511.43±7.64	<0.001
G_{Co400}	422.08±10.18	532.31±4.72	<0.001

$G_{control}$: normal rat, $G_{diabetes}$: diabetic rat, G_{gliben}: diabetic rat with glibenclamide treatment, G_{Co100}: diabetic rat and treatment with *Chromolaena odorata* extract 100mg/kg BW, G_{Co200}: diabetic rat and treatment with *C. odorata* extract 200mg/kg BW, G_{Co400}: diabetic rat and treatment with *C. odorata* extract 400mg/kg BW.

Immunohistochemical findings

Immunohistochemical examinations of the pancreas are given in Figure 3. The dark brown granules could be found spread in the middle region of the islet of Langerhans in $G_{control}$ (A). There was only a small amount of dark brown granules in the diabetic group ($G_{diabetes}$) (B) and a large number of irregular stained granules were found in G_{gliben} (C). The intensities of granules were increased with the higher dose of *C. odorata* extract (D-F) in which cells within G_{Co400} had higher intensity of brown granules.

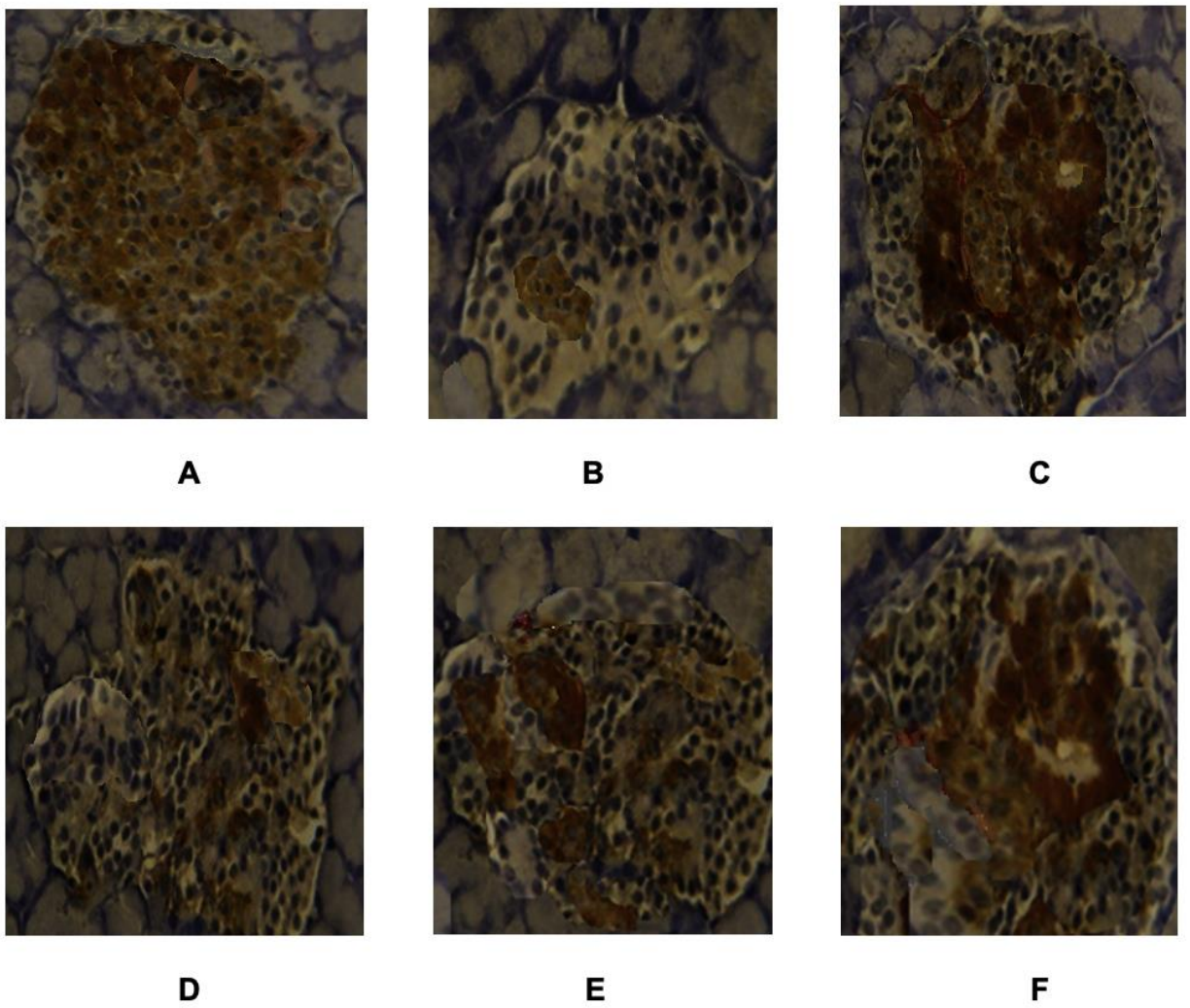

Figure 3. The Immunohistochemical staining of rat's insulin expression at 400 x magnification. The brown color shows the pancreatic beta cells that express insulin. A: normal rat, B: diabetic rat, C: diabetic rat with glibenclamide, D: diabetic rat with *Chromolaena odorata* extract 100mg/kg BW, E: diabetic rat with *C. odorata* extract 200mg/kg BW, F: diabetic rat with *C. odorata* extract 400mg/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[26, 27], resulting in limited endogenous insulin production, hyperglycemia, and weight loss[23]. It also affects the glucose level by destroying the insulin produced by beta cells in the islet of Langerhans[28, 29]. 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 $G_{diabetes}$, G_{gliben}, G_{Co100}, G_{Co200}, and G_{Co400} groups. 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[17]. Our data suggested that the administration of *C. odorata* leaves extract increased the body weight significantly in a dose-dependent manner. Rats within G_{Co400} had the most increase in body weight compared to the lower doses (i.e. G_{Co100} and G_{Co200}). This result is similar to another study that used the same dose (400 mg/kg BW)[20]. The increase in body weight was similar to animals that were treated with glibenclamide, suggesting that *C. odorata* extract could prevent the muscle wasting due to hyperglycemic status and improved the metabolic activity[17, 30].

Our data also suggested that *C. 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 *C. odorata* extract and this was similar in the group treated with glibenclamide. Previous studies have reported the hypoglycemic properties of *C. odorata* extract[31]. Leaves of *C. 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[32-35]. 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 of beta cells may explain the hypoglycemic activity of *C. odorata* as these mechanisms have been reported for lowering glucose level[36-39]. 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[40]. Our study indicated that the level of insulin increased in all *C. odorata* treated groups compared to those untreated animals; the highest rise was observed within group treated with the highest dose. This result suggested that the beta cells of treated animals have undergone a recovery state as showed in immunohistochemical examination (**Fig.3**). A previous study also suggested that *C. odorata* extract affected the extent of restoration of beta cell function[41] and *C. odorata* extract could regenerate the beta cells to almost similar to the normal control group[20]. 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. Immunohistochemical examination was assessed qualitatively only therefore further quantitative assessment will help to refine this preliminary finding. 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 *C. 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.

COMPETING INTEREST

The authors have declared that they have no conflict of interests.

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Authors' contributions

Conceptualization, HY, YY and FM; methodology, HY and YY; validation, HY, YY, FM and MF; formal analysis, HY and MF; investigation, HY, YY and FM; data curation, HY, YY and FM; writing—original draft preparation, HY, YY, FM and MF; writing—review and editing, HY and MF. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Harreiter J, Roden M. [Diabetes mellitus-Definition, classification, diagnosis, screening and prevention (Update 2019)]. Wien Klin Wochenschr. 2019; 131:6-15.
2. Turkmen K, Karagoz A, Kucuk A. Sirtuins as novel players in the pathogenesis of diabetes mellitus. World J Diabetes. 2014; 5:894-900.
3. Umar A, Ahmed QU, Muhammad BY, Dogarai BB, Soad SZ. Anti-hyperglycemic activity of the leaves of Tetracera scandens Linn. Merr. (Dilleniaceae) in alloxan induced diabetic rats. J Ethnopharmacol. 2010; 131:140-5.
4. Kitada M, Koya D. SIRT1 in Type 2 Diabetes: Mechanisms and Therapeutic Potential. Diabetes Metab J. 2013; 37:315-25.
5. Maharjan A, Karkee A, Prajapati M, Shrestha H, Manandhar Shrestha JT. Adverse Effects of Oral Hypoglycemic Agents and Adherence to them among Patients with Type 2 Diabetes Mellitus in Nepal. Journal of Lumbini Medical College. 2017; 5:34-40.
6. Ganesan K, Rana MBM, Sultan S. Oral Hypoglycemic Medications. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.; 2020.
7. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM. Herbal medicines for diabetes mellitus: a review. Int J PharmTech Res. 2010; 2:1883-92.
8. Hammeso WW, Emiru YK, Ayalew Getahun K, Kahaliw W. Antidiabetic and Antihyperlipidemic Activities of the Leaf Latex Extract of Aloe megalacantha Baker (Aloaceae) in Streptozotocin-Induced Diabetic Model. Evid Based Complement Alternat Med. 2019; 2019.
9. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. Biochim Biophys Acta. 2013; 1830:3670-95.
10. Kumar S, Kumar V, Prakash OM. Antidiabetic and hypolipidemic activities of Kigelia pinnata flowers extract in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed. 2012; 2:543-6.
11. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine. 1995; 2:137-89.
12. Naidoo K, Coopoosamy R, Naidoo G. Screening of Chromolaeana odorata (L.) King and Robinson for antibacterial and antifungal properties. Journal of Medicinal Plant Research. 2011; 5:4859-62.
13. Hanphanphoom S, Krajangsang S. Antimicrobial Activity of Chromolaena odorata Extracts against Bacterial Human Skin Infections. Modern Applied Science. 2016; 10:159-71.
14. Bhargava D, Mondal CK, Shivapuri JN, Mondal S, Kar S. Antioxidant Properties of the Leaves of Chromolaena odorata Linn. Journal of Institute of Medicine. 2013; 35:53-7.
15. Putri DA, Fatmawati S. A New Flavanone as a Potent Antioxidant Isolated from Chromolaena odorata L. Leaves. Evid Based Complement Alternat Med. 2019; 2019.
16. Hanh TT, Hang DT, Van Minh C, Dat NT. Anti-inflammatory effects of fatty acids isolated from Chromolaena odorata. Asian Pac J Trop Med. 2011; 4:760-3.
17. Omonije OO, Saidu AN, Muhammad HL. Anti-diabetic activities of Chromolaena odorata methanol root extract and its attenuation effect on diabetic induced

hepatorenal impairments in rats. Clinical Phytoscience. 2019; 5:23.
18. Balakrishna A, Josthna P. Evaluation of In Vitro Antioxidant Activity of Root Bark of Chromolaena Odorata-An Important Antidiabetic Medicinal Plant. Pharmacophore. 2014; 5:49-57
19. Onkaramurthy M, Veerapur VP, Thippeswamy BS, Reddy TN, Rayappa H, Badami S. Anti-diabetic and anti-cataract effects of Chromolaena odorata Linn., in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2013; 145:363-72.
20. Selvanathan J, Sundaresan S. Acute Toxicity Effects on Anti-Diabetic Activities of Chromolaena Odorata in Induced Wistar Albino Rats. Journal of Composition Theory. 2020; 13:206-22.
21. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012; 166:877-94.
22. Kumar S, Singh R, Vasudeva N, Sharma S. Acute and chronic animal models for the evaluation of anti-diabetic agents. Cardiovasc Diabetol. 2012; 11.
23. Mythili MD, Vyas R, Akila G, Gunasekaran S. Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microsc Res Tech. 2004; 63:274-81.
24. Leary S, Underwood W, Anthony R, Cartner S, Grandin T, Greenacre C, et al. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. AVMA American Veterinary Medical Association: Schaumburg, IL, USA. 2020.
25. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972; 97:142-5.
26. Li Y, Wei Y, Zhang F, Wang D, Wu X. Changes in the pharmacokinetics of glibenclamide in rats with streptozotocin-induced diabetes mellitus. Acta Pharmaceutica Sinica B. 2012; 2:198-204.
27. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 51:216-26.
28. Veerapur VP, Prabhakar KR, Thippeswamy BS, Bansal P, Srinivasan KK, Unnikrishnan MK. Antidiabetic effect of Ficus racemosa Linn. stem bark in high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats: a mechanistic study. Food Chem. 2012; 132:186-93.
29. Patel R, Shervington A, Pariente JA, Martinez-Burgos MA, Salido GM, Adeghate E, et al. Mechanism of exocrine pancreatic insufficiency in streptozotocin-induced type 1 diabetes mellitus. Ann N Y Acad Sci. 2006; 1084:71-88.
30. Ahn YH. A Journey to Understand Glucose Homeostasis: Starting from Rat Glucose Transporter Type 2 Promoter Cloning to Hyperglycemia. Diabetes Metab J. 2018; 42:465-71.
31. Adedapo AA, Io O, Adeoye A, Ofuegbe SO, Emikpe BO. Evaluation of the medicinal potential of the methanol leaf extract of Chromolaena odorata in some laboratory animals. Journal of Medicinal Plants Studies. 2016; 4:29-37.
32. Yang CY, Wang J, Zhao Y, Shen L, Jiang X, Xie ZG, et al. Anti-diabetic effects of Panax notoginseng saponins and its major anti-hyperglycemic components. J Ethnopharmacol. 2010; 130:231-6.
33. Kumari M, Jain S, Dave R. Babul (Acacia nilotica). Nutrition & Food Science. 2014; 44:119-26.
34. Ijioma S, Okafor A, Ndukuba P, Nwankwo A, Akomas S. Hypoglycemic, hematologic and lipid profile effects of Chromolaena odorata ethanol leaf extract in alloxan induced diabetic rats. Ann Biol Sci. 2014; 2:27-32.
35. Bamisaye F, Ajani E, Nurain I, Minari J. Medico-botanical investigation of siam weed (chromolaena odorata) used among the "ijebu" people of Ogun state, Nigeria. J Med Med Sci. 2014; 5:20-4.
36. Ogbonnia S, Mbaka G, Anyika E, Osegbo O, Igbokwe N. Evaluation of acute toxicity in mice and subchronic toxicity of hydro-ethanolic extract of Chromolaena odorata (L.) King and Robinson (Fam. Asteraceae) in rats. Agriculture and Biology Journal of North America. 2010; 1:859-65.
37. Adeneye AA, Agbaje EO. Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves ethanol extract of morinda lucida benth. in normal and alloxan- induced diabetic rats. African Journal of Biomedical Research. 2010; 11:65-71.
38. Bakirel T, Bakirel U, Keles OU, Ulgen SG, Yardibi H. In vivo assessment of antidiabetic and antioxidant activities of rosemary (Rosmarinus officinalis) in alloxan-diabetic rabbits. J Ethnopharmacol. 2008; 116:64-73.
39. Eddouks M, Jouad H, Maghrani M, Lemhadri A, Burcelin R. Inhibition of endogenous glucose production accounts for hypoglycemic effect of Spergularia purpurea in streptozotocin mice. Phytomedicine. 2003; 10:594-9.
40. Omonije OO, Saidu AN, Muhammad HL. Antioxidant and Hypolipidemic Effects of Methanolic Root Extract of Chromolaena odorata in Alloxan-induced Diabetic Rats. IJT. 2020; 14:63-70.
41. Grossman EJ, Lee DD, Tao J, Wilson RA, Park SY, Bell GI, et al. Glycemic control promotes pancreatic beta-cell regeneration in streptozotocin-induced diabetic mice. PLoS One. 2010; 5:e8749.