ACTIVITY NANOKIRINYUH (CHROMOLAENA ODORATA) LEAVES EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Objective: Diabetes Mellitus is a type of degenerative disease that is increasing every year in countries around the world. Diabetes Mellitus is a major cause of blindness, kidney failure, heart attacks, and stroke. Nanokirinyuh leaves have potential as an antidiabetic because they contain chemical compounds that have antioxidant activity. The purpose of this study was to determine activity of nanokirinyuh leaves as an antidiabetic.

Methods: Wistar rats as many as 24 animals were divided into 6 groups, namely the normal control group, positive control (glibenclamide 0.5 mg/Kg BW), negative control (alloxan 600 mg/BW rat), and nanochitosan kirinyuh leaves at a dose of 1225 mg/Kg BW rat, 450 mg/Kg BW rat and 675 mg/Kg BW rat were treated for 10 d. Percent decrease of level glucose was evaluated along with histopathological investigation in various experimental groups of rats. Data analysis using the One Way Anova test and continued LSD test.

Results: Level of Glucose at a dose of 675 mg/Kg BW rats showed the highest levels of the negative group and other dose groups. Pancreas histopathology test results showed that the group with a dose of 450 mg/Kg BW of rats had the lowest necrosis rate compared to the negative control group and other dose groups.

Conclusion: Nanokirinyuh leaves can reduce of level plasma glucose and necrosis in a histopathology test.

Keywords: Diabetes, Nanochitosan, Kirinyuh, Leaves

INTRODUCTION

Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. Between 2000 and 2016, there was a 5% increase in premature mortality from diabetes. In 2019, an estimated 1.5 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [1].

Diabetes is a metabolic disorder characterised by elevated blood sugar that results from defects in insulin production and/or insulin action, and impaired function in the metabolism of carbohydrates, lipids and proteins which leads to macro and microvascular complications [2]. Inflammation, endothelial dysfunction, and hypercoagulability are correlated to each other, playing an important role in the development of vascular complications in diabetic patients [3]. There is emerging evidence that oxidative stress makes a significant contribution to the progression of diabetes and its associated complications [2, 4]. Flavonoids in Kirinyuh leaves have a high antioxidant activity with a concentration inhibitor value (IC50) of 9.5671 ppm and has an antioxidant capacity of 49.037% [5]. Nanoparticles can be used as a conductor for more effective pharmaceutical compounds or drugs [6]. There is no one has reported antidiabetic activity of Chromolaena odorata loaded nanoparticles.

MATERIALS AND METHODS

Materials

The materials were nanokirinyuh leaves from Laboratory of Pharmacology Bakti Tunas Husada Institute of Health Science, glucose reagent kit GOD FS diaxys 8307269901, sodium CMC, Alloxan Aldrich 102103636, and sodium chloride, wistar rat from Bakti Tunas Husada animal house.

Experimental animals

The experimental protocol was approved by the Ethics Committee Bakti Tunas Husada Institute of Health Science No: 036/kepk-bth/07/07/2020. Twenty five wistar rats weighing 150-200 gram were obtained and acclimatised in one weeks. They were maintained under standard housing conditions with free access to a standard diet and water ad libitum during the experiment. Animals were divided randomly into 6 groups of 4 animals each namely normal group was administered at standard diet and water ad libitum, negative control group was administered intraperitoneal injection alloxan 170 mg/kg BW for 2 d, positive groups was administered alloxan intraperitoneal injection 170 mg/kg BW for 2 d and treated with glibenclamide 0.5 mg/Kg BW, dose 1 group was administered alloxan 170 mg/KgBW intraperitoneal injection for 2 d and treated with 225 mg/Kg BW of nanokirinyuh leaves extract for 14 d, dose 2 group was administered alloxan 170 mg/Kg BW intraperitoneal injection for 14 d and treated with 450 mg/Kg BW rat of nanokirinyuh leaves for 14 d and dose 3 group was administered alloxan 170 mg/KgBW intraperitoneal injection for 14 d and treated with 675 mg/Kg BW rat of nanokirinyuh leaves extract for 14 d.

Biochemical analysis

The rats were anaesthetised with diethyl ether and 2-3 ml of blood samples was collected by orbital venous sinus. The samples were allowed to clot and centrifuged at 3000 rpm at 30 °C for 10 min and the separated serum was used for the following biochemical estimations using commercially available kits: Glucose GOD FS (Diasys Diagnostic System Germany).

Histopathology of rat pancreas

After blood sampling, all the animals were sacrificed by cervical dislocation under deep anesthesia and subjected to a complete necropsy followed by histopathology. The rat pancreas were identified and carefully dissected out for histopathological examination. After rinsing in normal saline, sections were taken from each harvested pancreas, fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and embedded in paraffin wax. The 5-6 μm sections were prepared using a rotary microtome and stained with haematoxylin and eosin dye for...
Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant intergroup differences of each parameter. A p value < 0.05 was considered statistically significant and continued the LSD test.

RESULTS

Phytochemical screening aims to find out what group of compounds are found in phytochemical screening aims to find out what groups of compounds were found in *Chromolaena odorata* simplicia and extract. The result of phytochemical screening can be seen in table 1.

| Phytochemical       | Simplicia | Extract |
|---------------------|-----------|---------|
| Flavonoids          | +         | +       |
| Polyphenols         | +         | +       |
| Tannins             | -         | -       |
| Saponins            | -         | -       |
| Steroids            | +         | -       |
| Sesquiterpenoids    | +         | +       |
| Monoterpenoids      | +         | +       |
| Quinones            | +         | +       |

The results in table 1 shows that both simplicia and extract contain flavonoids, polyphenols, sesquiterpenoid, monoterpenoid and quinone compounds. Steroids exist on simplicia but they were not found on extracts.

| Group                | Level glucose before (mg/dl) | Level glucose after (mg/dl) | Percent decrease (%) |
|----------------------|-----------------------------|-----------------------------|----------------------|
| Dose 225 mg/Kg BW    | 246.032                     | 240.136                     | 2%                   |
| Dose 450 mg/Kg BW    | 267.760                     | 230.619                     | 14%                  |
| Dose 675 mg/Kg BW    | 252.303                     | 176.028                     | 30%                  |
| Normal               | 150.147                     | 150.433                     | 0%                   |
| Negative control     | 283.476                     | 277.690                     | 2%                   |
| Positive control     | 262.946                     | 232.651                     | 12%                  |

Fig. 1: Pancreatic histopathology, a=Dose 225 mg/ Kg BW; b= Dose 450 mg/ Kg BW; c=Dose 675 mg/ Kg BW; d =Normal; e= Negative; f=Positive
Based on histopathological results, the negative group had the most pancreatic necrosis cells compared to the other treatment groups, a dose of 450 mg/Kg BW had the least necrosis cells compared to the other groups but not less than the normal group. This is because the normal group was not given alloxan induction treatment. Alloxan can induce pancreatic damage have been demonstrated with structural and functional alterations such as disorganization of pancreatic architecture, and depletion of insulin producing cells. The cytoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration leading to rapid destruction of b-cells and necrosis cell [7, 8].

**DISCUSSION**

Free radicals and other 'reactive species' are involved in many human diseases and that the increased formation of 'free radicals' accompanies tissue injury. Free radical reactions are essential for host defence mechanisms as with neutrophils, macrophages and other cells of the immune system [9]. Oxidative stress is produced under diabetes conditions and is likely involved in the progression of pancreatic beta-cell dysfunction found in diabetes [10]. Blood glucose levels in the treatment group increased>200 mg/dl after being induced by alloxan. Alloxan can destroy the insulin secreting cells of the pancreas resulting in hypoinsulinemia and hyperglycemia [11]. Flavonoids can reduce the number of lesions formed by increasing the amount of exogenous antioxidants in the body to compensate for endogenous antioxidants and natural antioxidant activity that can neutralize or capture free radicals [5].

Chitosan is a natural polymer which has characteristics such as nontoxic, mucoadhesive, biodegradable, biocompatible, a low level of immunogenicity and can be prepared into nanoparticles in mild conditions. Therefore, it is suitable for delivery systems of natural extracts [12]. Chitosan has properties similar to dietary fiber in that it can not be digested by mammal digestive enzymes, therefore chitosan does not have caloric value. In addition, chitosan can also be beneficial in metabolism of fat and glucose control. This substance is a biocompatible and biodegradable polymer with low toxicity [13]. Chitosan stimulates the secretion of leptin and adiponectin from adipose tissues. In the liver, chitosan enhances the phosphorylation of AMPK and down regulates PEPCK and adinopectin from adipose tissues. In the liver, chitosan enhances the activity of ethanolic extract of tubers of dioscorea alata in alloxan treated rats for induction of diabetes. J Basic Appl Zool. 2013;66(4):169-79. doi: 10.1016/j.jbazar.2013.07.003.

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**CONCLUSION**

Levels of glucose at a dose of 675 mg/Kg BW rats showed the highest levels of the negative group and other dose groups. Pancreas histopathology test results showed that the group with a dose of 450 mg/Kg BW of rats had the lowest necrosis rate compared to the negative control group and other dose groups. Nanochitosan kirinyuh leaves can reduce of level plasma glucose and necrosis in a histopathology test.

**FUNDING**

Nil

**AUTHORS CONTRIBUTIONS**

All the authors contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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