GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Hypoglycemic activity of ethanol leaf extract of Grona trifloral in alloxan induced diabetic mice

Sonne Ikechukwu Mbagwu, Daniel Ikechukwu Oraekei * and Loveth Chioma Onah

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2023, 24(03), 028–040

Publication history: Received on 16 July 2023; revised on 31 August 2023; accepted on 03 September 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.24.3.0347

Abstract

Diabetes is metabolic disease characterized by elevated level of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. Diabetes can be type 1, type 2 or gestational. Attention is now being diverted to natural remedies such as herbs. This study investigated the anti-diabetic properties of *Grona trifloral* ethanol extract using mice diabetic model. Ethanol extract of the leaf of *Grona trifloral* was prepared by cold maceration and analyzed for its phytochemical components. The acute toxicity of the extract was determined. Diabetes was induced by intraperitonial injection of alloxan monohydrate (150 mg/kg). A total of 25 mice with evidence of diabetes were randomized into 5 groups of five rats each and treated as follows: Group 1 (normal control), group 2 (diabetic control), group 3 (metformin, 100 mg/kg) and groups 4 and 5 (1/5 and 1/20 of leaf extracts LD_{50}) respectively. Fasting blood glucose levels were determined using Glucometer on stipulated days. At the end of the experiments, LD50 > 5,000 mg/kg body weight and the phytochemicals present in *Grona trifloral* were alkaloids, flavonoids, reducing sugars, proteins, tannins, triterpenoids and glycosides. There were significant ($p < 0.05$) dose dependent percentage decreases in glucose levels in the groups treated with *Grona trifloral* leaf extract. In conclusion, *Grona trifloral* ethanol leaf extract had dose dependent increase in anti-hyperglycemic effects and at the highest tested dose of 500 mg/kg body weight, *Grona trifloral* hypoglycemic activities surpassed that of metformin (a standard anti-diabetic drug).

Keywords: Alloxan; Diabetic mice; *Grona trifloral*; Hypoglycemic activity; Phytochemicals

1. Introduction

Diabetes is a chronic, metabolic disease characterized by elevated level of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves [1]. Diabetes can be type 1 or type 2 and the most common is type 2 diabetes which usually occur in adults. Type 2 diabetes occurs when the body becomes resistant to insulin or does not make enough insulin. On the other hand, type 1 diabetes also known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself. People living with type 1 diabetes need insulin administration regularly because there is no cure for this type of diabetes. When the blood sugar is managed successfully, type 1 diabetics can subdue serious complications such as ketoacidosis, nerve damage, ocular damage, renal failure, cardiovascular diseases notably hypertension and stroke among others. A person living with type 2 diabetes may or may not require insulin. Many of the cases are effectively managed with medication, exercise and diet. Type 2 diabetes can occur in both children and adults and its most important risk factors include: age 45 or older, overweight and family history. Another type of diabetes that needs mentioning is gestational diabetes which occurs during pregnancy when a pregnant woman becomes less sensitive to insulin. Individuals who are overweight going into their pregnancy have an elevated risk of developing the condition with the likelihood of developing type 2 diabetes later in their life. Gestational diabetes can be managed by the individuals engaging in activities, monitoring the growth and development of the fetus, adjusting their diet and monitoring blood sugar levels.

* Corresponding author: Daniel Ikechukwu Oraekei; Email: di.oraekei@unizik.edu.ng

Consequences of gestational diabetes include: elevated risk of developing high blood pressure during pregnancy, premature birth, increased birth weight, and increased risk of the baby developing type 2 diabetes later in life. The rational for the treatment of diabetes mellitus is to maintain fasting blood sugar level of ≤ 126 mg/dL or random blood sugar level of ≤ 200 mg/dL. Currently used oral anti-diabetic agents such as sulphonylureas, biguanides (metformin) and the thiazolidinediones (rosiglitazone, pioglitazone) commonly target fasting hyperglycaemia and have limited additive effects on postprandial glycaemia. In contrast, alpha-glucosidase inhibitors can reduce postprandial hyperglycaemia but gastrointestinal side effects restrict their use [2]. A more recent approach is to individualize treatment according to the individual's glycemic targets and to optimize the treatment [3]. However, anti-diabetic drugs are well known for their limitations and adverse reactions. According to a certain study, the limitations of insulin-dependent oral anti-diabetic agents were reported. The main drawback of metformin, sulfonylureas, gliptins and to a lesser extent-glitazones is durability. No drug per se is able to maintain stable blood glucose control for years [4]. According to the researchers, the main adverse effect of metformin is gastrointestinal discomfort and major concerns related to the use of sulfonylureas are hypoglycemia and weight gain. The use of pioglitazone was associated with an increased risk of bladder cancer, edema, heart failure, weight gain, and distal bone fractures in postmenopausal women. The most common adverse reactions associated with glucagon-like peptide-1 agonists are gastrointestinal discomfort that sometimes leads to treatment discontinuation.

Based on these limitations and adverse effects of standard anti-diabetic drugs, attention is now being diverted to natural remedies such as the use of herbs following the anticipation that herbal drugs have less adverse effects among being cheap and readily available. The beneficial potential of these herbs has been attributed to the numerous phytochemicals in them which can act individually or in synergy to achieve optimal therapeutic effects [5]. Diabetes mellitus is becoming more prevalent in developing countries and this may be as a result of the nutritional lifestyle in which majority of the citizens are inclined to carbohydrates such as rice, yam, cassava, maize among others. There is a growing need for less expensive, more safe, and more readily available alternative which is achieved through the use of herbal drugs. We therefore tested the effectiveness of *Grona trifloral* on diabetes using mice model.

Grona trifloral has been in use locally in many parts of Anambra state of Nigeria for controlling elevated blood sugar levels. However, no study has been done on this herb as to confirm its potentials in the management of diabetes mellitus. In this study therefore, we evaluated the hypoglycemic activity of ethanol leaf extract of *Grona trifloral* in alloxan induced diabetic mice. Studies have shown that all countries, irrespective of their developmental stage, face an increasing burden of non-communicable diseases including diabetes mellitus. There have been an increase in the prevalence of diabetes mellitus which is related to factors such as urbanization, obesity, overweight, and hypertension. Individuals in these categories have higher chances of getting diabetes mellitus [6]. Diabetes mellitus when prolonged has serious metabolic consequences which include accelerated atherosclerosis, chronic kidney disease and blindness {7]. These pose enormous burden on patients with diabetes mellitus and on the public health system. In the year 2000, according to the World Health Organization (WHO), at least 171 million people worldwide suffer from diabetes, or 2.8% of the population [8]. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will more or less double. The majority of type 1 diabetes is of the immune mediated nature, where beta cell loss is a T-cell mediated autoimmune attack [9]. There exists only a weak genetic link in the etiology of this form of diabetes. Type 1 diabetes can conveniently be treated with insulin. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children [10]. A study done on the epidemiology of type 1 diabetes noted that greater than 350-fold difference in the incidence among the 100 populations worldwide was reported with age-adjusted incidences ranging from a low of 0.1/100,000 per year in China and Venezuela to a high of 36.5/100,000 in Finland and 36.8/100,000 per year in Sardinia. The lowest incidence (<1/100,000 per year) was reported in the populations from China and South America and the highest incidence (>20/100,000 per year) was reported in Sardinia, Finland, Sweden, Norway, Portugal, the UK, Canada, and New Zealand [10]. Susceptibility of type 1 diabetes appears to involve a multifactorial genetic linkage but only 15–20% of patients have a positive family history [11]. Type 2 diabetes mellitus is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases become absolute. The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity [12]. It occurs more frequently in women with prior gestational diabetes mellitus, impaired glucose tolerance or "prediabetes" postpartum and in individuals with hypertension or dyslipidemia [13]; frequency also varies in different racial/ethnic subgroups. It is not known what make the body tissues to become unresponsive to insulin despite the insight that the process of defective responsiveness almost certainly involves the insulin receptor in the cell membranes. Diabetes mellitus due to known specific defect are classified separately. There are numerous theories as to the exact cause and mechanism in type 2 diabetes [14]. Central obesity (fat concentrated around the waist in relation to abdominal organ, but not subcutaneous fat) is known to predispose individuals to insulin resistance. Abdominal fat is especially active hormonally, secreting a

group of hormone called adipokines that may possibly impair glucose tolerance. Adipokines and cytokines may decrease the insulin sensitivity of tissues and induce inflammation and development of chronic complications [15] Obesity is found in approximately 55% of patients diagnosed with type 2 diabetes [16]. Other factors include aging (about 20% of elderly patients in North America have diabetes) and family history (type 2 diabetes is much more common in those with close relatives who have had it). A positive correlation has been found between the concentration in the urine of bisphenol A, a constituent of polycarbonate plastic, and the incidence of type 2 diabetes [17]. A given individual may have more resistance or more B cell deficiency, and the abnormalities may be mild or severe. Although insulin is produced by the B cells in these patients, it is inadequate to overcome the resistance, and the blood glucose rises. Dehydration in untreated and poorly controlled individuals with type 2 diabetes can lead to a life-threatening condition called "non-ketotic hyperosmolar coma". In this condition, the blood glucose may rise to 6–20 times the normal range and an altered mental state develops or the person loses consciousness thus requiring urgent medical care and rehydration. Another type of diabetes mellitus designated as type 3 refers to many other specific causes of hyperglyceamia such as pancreatic diseases, drugs, hormones, genetics, and other abnormalities [1]. An example of hormone induced diabetes is Diabetes insipidus, which results from abnormalities in antidiuretic hormone secretion. Gestational diabetes mellitus which is defined as any abnormality in glucose levels noted for the first time during pregnancy is diagnosed in approximately 4% of pregnancies while the prevalence may range from 1–14% of all pregnancies depending on the population and the method of screening [18]. During pregnancy, the placenta and placental hormones create an insulin resistance that is most pronounced in the last trimester. Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20%-50% of affected women develop type 2 diabetes later in life [13]. Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. A study completed in the United State found that more American women are entering pregnancy with pre-existing diabetes. The researchers recorded that approximately 0.9% of the 4,000,000 births in the United States are complicated by preexisting diabetes and this increases the risk of adverse maternal and neonatal outcomes, such as preeclampsia, caesarian section, pre-term delivery, macrosomia and congenital defects [19]. The prevalence of gestational diabetes mellitus was estimated to have increased by more than 30 % within one or two decades in a number of countries including developing countries [20]. This is particularly problematic as diabetes raises the risk of complication during pregnancy as well as increasing the potential that the children of diabetic mothers will also become diabetic in future. Risk assessment for diabetes is suggested starting at the first prenatal visit. High risk individuals should be screened immediately. Screening for diabetes may be deferred in lower risk women until the 24th to 28th week of gestation. The recommendations for screening women at risk of diabetes for first and subsequent trimester at 24-28 weeks is made by universal glucose tolerance testing using the criteria of 75 g OGTT and fasting 5.1mmol/l, 1 hour 10.0mmol/l, and 2 hour 8.5mmol/l as diagnosis of gestational diabetes mellitus [21]. It was documented that diabetes mellitus are genetically determined. Over 20 regions in the human genome are associated with Type 1 diabetes, but makes little contribution to overall susceptibility to Type 1 diabetes. The strongest linkage with Type 1 diabetes is shown by the human leucocyte antigen (*HLA*) gene cluster in the major histocompatibility complex (*MHC*) located on chromosome 6p21. This is due to the multiple genes and extreme polymorphism at those HLA region loci [22]. HLA antigens are cell-surface glycoproteins that play a crucial role in presenting auto antigen peptide fragments to T lymphocytes and thus initiate an auto immune response. Type 2 diabetes is polygenic, with different combinations of gene defects [23]. Under normal circumstances, liver and muscle cells take up glucose and convert any excess to the storage form glycogen. If insulin is absent, these cells will not be able to take up glucose which is required as an energy source by the cells. When serum glucose is elevated to very high levels, the intracellular concentration of glucose in many types of cells, notably nerve, epithelia, and kidney cells, also rises greatly. This so-called “spiking” of intracellular glucose levels is believed to cause most of the serious complications of diabetes. At abnormally high levels of glucose, the enzyme aldose reductase is activated. This enzyme convents the excess glucose into the corresponding sugar alcohol, known as sorbitol. Sorbitol, like all sugar alcohol, is so polar that it is unable to cross any biological membrane, and so is metabolized to fructose only very inefficiently. Thus, sorbitol concentration in affected cells rises a little with each glucose spike, until finally the cell osmolality becomes so high that the cells ceases to function or ruptures. Almost all the difficulties encountered in diabetes arise from this phenomenon, and this explains why prevention of these glucose spikes occupies such a prominent place in modern diabetes treatment. It has been suggested that there may be a glycemic threshold for diabetes complications. According to this research, hyperosmotic complications are unlikely if hemoglobin (termed glycated hemoglobin or HbAIc) levels remains below 8.1%. Complications of diabetes might be prevented if one had drugs which inhibited aldose reductase such as Zenarestat and Zopolrestat. Diabetic complications include: Ocular problems as in diabetic retinopathy; diabetic nephropathy due to excess sorbitol which causes lesions in small blood vessels similar to those seen in the eye; atherosclerosis and other Vascular Complications which are associated with increased risk of stroke, heart attack, and other complications. Compromised circulation in the legs can lead to non-healing leg ulcers with gangrene, sometimes requiring amputation of the affected limbs. Skin infections, especially those due to *Candida albicans*, are also commonly observed in diabetics. The classical triad of diabetes

symptoms include polyuria, polydipsia, and polyphagia, which are, respectively, frequent urination, increased thirst and consequent increased fluid intake. Increase appetite symptoms may develop quite rapidly (weeks or months) in type I diabetes particularly in children. Type I diabetes may also cause a rapid and significant weight loss (despite normal or even increased eating) and irreducible fatigue. However, in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent. These symptoms except weight loss manifest in type 2 diabetes in patients whose diabetes is poorly controlled. Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following: Fasting plasma glucose level ≥ 7.0 mmol/L (126 mg/dL); Plasma glucose ≥ 11.1 mmol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test; Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/L (200 mg/dL); Glycated hemoglobin (Hb A1C) ≥ 6.5% [1]. A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/L) is considered diagnostic for diabetes mellitus. People with fasting glucose levels from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance [21]. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease. Type 2 diabetes risk can be reduced in many cases by making changes in diet and increasing physical activity. The American Diabetes Association (ADA) recommends maintaining a healthy weight, getting at least 21/2 hours of exercise per week, having a modest fat intake, and eating sufficient fiber [24]. Some studies have shown delayed progression to diabetes in predisposed patients through prophylactic use of metformin [25]. Rosiglitazone [26]., or valsartan; in a study to evaluate the effects of valsartan on the incidence of diabetes and cardiovascular events, the researchers concluded that among patients with impaired glucose tolerance and cardiovascular disease or risk factors, the use of valsartan for 5 years, along with lifestyle modification, led to a relative reduction of 14% in the incidence of diabetes but did not reduce the rate of cardiovascular events [27]. Diabetes mellitus is currently a chronic disease without a cure, and medical emphasis must necessarily be on managing/avoiding possible short term as well as long-term diabetes-related problems. There is an exceptionally important role for patient education, dietetic support, sensible exercise, self-monitoring of blood glucose, with the goal of keeping both short-term blood glucose levels, and long term levels as well, within acceptable bounds. Careful control is needed to reduce the risk of long term complications. This is theoretically achievable with combinations of diet, exercise and weight loss (type 2) various oral diabetic drugs (type 2 only), and insulin use (type 1 and for type 2 not responding to oral medication, mostly those with extended duration diabetes). In addition, given the associated higher risks of cardiovascular disease, lifestyles modifications should be undertaken to control blood pressure and cholesterol by exercising more, stopping smoking, controlling ones diet, and taking antihypertensive drugs. Many type I diabetes treatment include combination use of regular or neutral *protamine* hagedorn (NPH) insulin, and/or synthetic insulin analogs. These insulin preparations has been developed to partially compensate for the inability to deliver timely exogenous insulin directly to the portal/intrapancreatic circulation. Several recent technological advances help addressing these goals, including the rapid progress in insulin pumps, continuous glucose sensors, and ultimately the artificial pancreas closed-loop technology and the recent start of dual-hormone therapies [28]. The treatment of diabetes mellitus takes 3 main forms: diet, exercise and lifestyle changes; Insulin replacement therapy; and the use of oral hypoglycaemic agents. Currently, there are ten classes of orally available pharmacological agents to treat type 2 diabetes mellitus. These include: sulfonylureas, meglitinides, metformin (a biguanide), thiazolidinediones (TZDs), alpha glucosidase inhibitors, dipeptidyl peptidase IV (DPP-4) inhibitors, bile acid sequestrants, dopamine agonists, sodium-glucose transport protein 2 (SGLT2) inhibitors and oral glucagon like peptide 1 (GLP-1) receptor agonists [29]. The researchers reported additional use of glucagon like peptide 1 (GLP-1) receptor agonists, dual GLP-1 receptor and GIP receptor agonists, and amylin which can be administered by injection. Medications from these distinct classes of pharmaceutical agents may be used as monotherapy or in a combination of two or more drugs from multiple classes with different mechanisms of action. However, since time immemorial, patients with non-insulin dependent diabetes mellitus (NIDDM) have been treated orally in folk medicine with a variety of plant extracts [30]. We therefore tested our plant *Grona trifloral* to evaluate its relevance in this modern day treatment of diabetes mellitus.

Grona trifloral is a much branched, mat-forming, prostrate, annual to perennial herb producing stems 8-50 cm long from a woody taproot. It behaves as either annual or perennial depending on distribution of rainfall. The stems are strongly branched and frequently root at the nodes. The plant is gathered from the wild for local medicinal uses. *Grona trifloral* has antipyretic, antiseptic and expectorant properties and can therefore be used as a phytomedicine [31]. A decoction of the plant is commonly used to treat diarrhea and dysentery; to quench thirst and as a mouthwash; and the crushed plant, or a poultice of the leaves, is applied externally on wounds, ulcers, and for skin problems in general. The whole plant is used medicinally for inducing sweat and promoting digestion. Currently, no research has been reported

on the Pharmacological uses of *Grona trifloral.* We therefore carried out this novel study which is aimed at evaluating the anti-diabetic potentials of the ethanol leaf extract of *Grona trifloral* in alloxan induced diabetes in mice model.

2. Material and methods

2.1. Materials

2.1.1. Equipment

Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven (Gallenkamp, England), Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropippete (Finnipipette® Labsystems, Finland) and Intubation tubes, glucometer, amber colored bottles, filter paper, refrigerator.

2.1.2. Chemicals, reagents and drugs

The following chemicals and drugs were used in the course of this research. Alloxan monohydrate (Sigma China), metformin (NGC, Nigeria), ethanol (JHD, Guangdong Guanghua Schi-Tech. Ltd China), Formaldehyde 40% w/v, chloroform, tween-80, Dragendoffs reagent, Mayers reagent, Wagners reagent, Hagers reagent.

2.1.3. Plant materials

Fresh leaves of *Grona trifloral* was collected from school of pharmacy Agulu. The plant was authenticated by a Taxonomist at the Department of Botany, Nnmadi Azikiwe University, Awka, Anambra State, Nigeria.

2.1.4. Animals

A total of 25 albino Swiss mice, average weight (18 - 35 g) were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. They were housed in clean plastic cages, supplied clean drinking water and fed with commercial pelleted feed (Guniea Feed®, Nigeria in the animals' house, School of Pharmacy, Agulu. Animals were handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals (Pub No. 85-23, revised 1985). Ethical approval for handling of laboratory animals was obtained from the Animal Research and Ethics Committee, Nnamdi Azikiwe University, Awka. The ethical approval number is NAU/AREC/2023/00077.

2.2. Methods

2.2.1. Extraction of plant material

Fresh leaves of *Grona trifloral* were gently dipped in a big bowel containing cold water to remove sands and particles. The leaves were air-dried for a period of two weeks at room temperature. The dried leaves of *Grona trifloral* was ground with electric blender and kept in a clean air tight amber colored bottle. A quantity of 1,000 g of the powered leaves material was weighed out using a weighing balance and 500 ml of 80% ethanol was used to soak it (maceration) and was agitated continuously. The soaked mixture was kept for 48 hours. The filtrate was recovered using filter paper and was concentrated to dryness using water bath at 40 °C. The extract was stored properly in a refrigerator prior to use. The percentage yield of the extract was calculated using the following formula.

$$\% \text{ yield} = (\text{mass of dry extract}/1{,}000 \text{ g}) \times 100$$

2.2.2. Phytochemical analysis

The extract was tested for the presence of various plant constituents like Alkaloids, Flavonoids, Reducing sugars, Saponins, Proteins, Tanins, Amino acids, Steroids, Triterpenoids and glycosides [32, 33, and 34].

Tests for Alkaloids

To small amount of the extract sample was added few drops of dilute hydrochloric acid, mixed and filtered. The following tests for alkaloids were carried out with the filtrates.

- **Mayer's reagent**: A portion of the filtrate was treated with Mayer's reagent and observed. The presence of yellow or creamy precipitate indicated the presence of alkaloids.

- **Dragendoff's reagent**: A portion of the filtrate was treated with Dragendoff's reagent and observed. The presence of a reddish-brown precipitate indicated the presence of alkaloids.
- **Wagner's reagent**: A portion of the filtrate was treated with Wagner's reagent and observed. The presence of a reddish-brown precipitate suggested the presence of alkaloids.
- **Hager's reagent**: A portion of the filtrate was treated with Hager's reagent and observed. The presence of yellow precipitate indicated the presence of alkaloid.
- **Test for flavonoids**
- **Lead acetate test**: The filtrate was treated with a few drops of lead acetate solution. Formation of yellow precipitate indicated the presence of flavonoids.
- **Alkaline reagent test**: The filtrate was treated with a few drops of sodium hydroxide. Formation of intense yellow color, which became colorless on addition of few drops of dilute acid indicated the presence of flavonoids.

Test for reducing sugar (Carbohydrates)

- **Benedict test**: Small quantities of the test samples in water were treated with Benedict solution and heated to boiling in water bath. None appearance of brick red precipitate indicated the absence of reducing sugar.
- **Fehling's test**: Small quantities of the test samples in water were treated with equal volumes of Fehling's A and Fehling's B solution and heated in a water bath for 10 minutes. Formation of red precipitate indicated the presence of a reducing sugar.

Test for Saponins

Frothing test: The filtrate was treated with small amount of water and shaken for about 15 minutes in a graduated cylinder. None formation of a stable 1 cm foam layer indicated the absence of Saponins.

Test for Proteins

- **Million's test:** To a 1 ml of test solutions, treated with sulphuric acid was added a small amount of million's reagent and boiled. The sample was observed for formation of white precipitate which turns red after warming and this indicated the presence of protein.
- **Precipitation test:** The test solutions gave white colloidal precipitate with the following reagents: 5% CuSO4 and 5% Lead acetate and this indicated the presence of proteins.

Tests for Tannins

- **Ferric Chloride test:** To 2 ml of the filtrate was added 5% dilute ferric chloride solution, a violet color formation indicated the presence of tannins**.**

Test for Amino acids

- **Ninhydrin test:** To 3 ml of the filtrate, three drops of 5% v/w lead acetate solution were added and heated to boiling in a water bath for 10 minutes. No change in color of the solution to purple or blue indicated the absence of amino acids.

Test for Steroids and Triterpenoids

- **Salkowski test:** Small amount of chloroform was added to 5 ml of the filtrate and few drops of concentrated sulphuric acid added. The mixture was shaken well and kept aside for some time and observed. None red color appearance indicated the absence of steroids and appearance of yellow color in the lower layer indicated the presence of triterpenoids.

Test for Glycosides

- **General test:** This was done using the Fehling's method of test for reducing sugar. After the Fehling's method as explained above, a portion of the sample was hydrolyzed with dilute sulphuric acid in separate test tubes. The increase in color intensity indicated the presence of glycosides.

2.2.3. Acute toxicity study (LD_{50} determination)

Acute toxicity, LD_{50} test was carried out using the method of Lorke (1983) [35] using a total of 13 mice, average weight of 30 g in two phases. In the first stage, the animals were divided into 3 groups of 3 mice each, and the extract was

administered at three dose level (10, 100 and 1000 mg/kg body weight). The animals were then monitored for 24 hours. Absence of deaths in the first phase led to the use of 2000, 3000, 4000 and 5000 mg/kg body weight of extract for 4 groups of 1 animal each. Animals were examined again for another 24 hours. Any number of death (s) was noted for each group and the LD_{50} calculated as follows;

$$LD_{50} = (H \times L)^{1/2}$$

H = Highest dose that resulted to no mortality
L = Lowest dose that resulted to mortality

2.2.4. Diabetes studies

Anti-diabetic study of *Grona trifloral* was carried out. A total of 25 adult albino mice were used. They were grouped into 5 groups of 5 mice per group. The animals were starved for 18 hours having access to drinking water alone prior the experiment. Hyperglycemia was induced using the method described by Osasenaga (2017) [36]; with a single intraperitoneal administration of 150 mg/kg body weight of *Alloxan monohydrate*; followed by oral administration of 2,000 mg/kg body weight glucose. The alloxanized mice were kept for 2 days with free access to feed and water for hyperglycaemia to develop. Baseline fasting blood glucose levels were determined using one Touch Glucometer (Lifescan, USA). Mice with glucose levels above 200 mg/kg were recruited for the study.

2.3. Experimental design

A total of 25 animals consisting of 20 diabetic mice and 5 non-diabetic mice were randomized into 5 groups. Group 1 served as normal control and received 10 ml/kg body weight of distilled water orally; group 2 received 100 mg/kg body weight of metformin orally; group 3 mice were treated 100 mg/kg body weight of crude extract; group 4 were treated 250 mg/kg body weight of crude extract; and group 5 were treated with 500 mg/kg body weight of crude extract. Baseline blood glucose level (zero hour) of animals in all the groups were taken with the aid of glucometer. Daily administration of metformin and extract were carried out for a period of 14 days (2 weeks) and blood glucose level of all the groups were measured at Day 3, Day 7 and Day14 with the aid of glucometer machine. At the end of 2 weeks, animals were fasted overnight and final blood glucose level taken. Animals were sacrificed and their pancreas collected for further histology studies.

2.4. Statistical Analysis

Results gathered from the study were analyzed using statistical package for social sciences (SPSS-20). Results were presented as Mean ± Standard Error of Mean (SEM). Raw data was subjected to one-way analyses of variance (ANOVA) followed by post hoc turkey's test. $P < 0.05$ was considered to be statistically significant.

3. Results

Table 1 Results of phytochemical analysis of *Grona trifloral* ethanol leaf extract

Tests		**Availability**
Alkaloids	Mayer's	+
	Dragendorf's	+
	Wagner's	+
	Hager's	+
Flavonoids	Lead acetate test	+
	Alkaline reagent test	+
Reducing sugars	Benedict's test	-
	Fehling's test	+
Saponins	Frothing test	-
Proteins	Millon's test	+

	Precipitation test	+
Tannins	Ferric Chloride test	+
Amino acids	Ninhydrin test	-
Steroids	Salkowski test	-
Triterpenoids	Salkowski test	+
Glycosides	General test	+

Key: + = present; - = absent

Table 2 Results of acute toxicity study of *Grona trifloral* ethanol leaf extract

Groups	Doses (mg/kg body weight)	Number of rats	Number of deaths
1	10	3	Nil
2	100	3	Nil
3	1,000	3	Nil
4	2,000	1	Nil
5	3,000	1	Nil
6	4,000	1	Nil
7	5,000	1	Nil

According to the results in table 2, no death was recorded up till 5,000 mg/kg body weight; LD50 was > 5,000 mg/kg body weight.

Table 3 Mean blood glucose level after treatments

MEAN ± SEM (mg/kg) blood glucose level				
Groups	**Basal**	**Day 3**	**Day 7**	**Day 14**
10 ml/kg DW	203.33 ± 1.20	300.33 ± 35.14	276.33 ± 15.94	273.67 ± 17.82
100 mg/kg metformin	230.67 ± 20.22	196.00 ± 9.84	117.67 ± 24.06	59.50 ± 2.73
100 mg/kg crude extract	339.33 ± 28.17	268.67 ± 33.84	256.67 ± 34.64	204.33 ± 40.76
250 mg/kg crude extract	213.67 ± 8.37	198.33 ± 12.57	181.33 ± 5.24	100.67 ± 10.2
500 mg/kg crude extract	237.33 ± 23.62	155.33 ± 26.74	103.33 ± 25.37	57.37 ± 8.29

Table 4 Percentage decrease in blood glucose level

Percentage decrease in blood glucose level (%)			
Groups	**Day 3**	**Day 7**	**Day 14**
10 ml/kg DW	0	0	0
100 mg/kg metformin	15.02	48.99	74.21
100 mg/kg crude extract	20.82	24.36	39.78
250 mg/kg crude extract	7.18	15.13	52.89
500 mg/kg crude extract	34.55	56.46	75.83

4. Discussion

The results of the phytochemical analysis showed the presence of alkaloids, flavonoids, reducing sugars, proteins, tannins, triterpenoids and glycosides while saponins, amino acids and steroids were absent. Several researches had shown that phytochemicals have the potentials to decrease blood glucose levels and have been used widely in the treatment of diabetes mellitus. In one of such studies, medicinal plants were reiterated to play a fundamental part in health sectors via the management of different infectious diseases because of their wide sources of bioactive phytochemicals. In the study which was the literature-based analysis of alkaloids from medicinal plants in preventive or treatment approaches to diabetes. The researchers reported that alkaloids showed anti-diabetic activity through the inhibition of enzymes (*α*-amylase, *α*-glucosidase, aldose reductase, dipeptidyl peptidase-IV, and protein tyrosine phosphatase-1B); inhibition of advanced glycation end products; increment of insulin secretion and its sensitivity; enhancement of glucose uptake; and their antioxidant ability [37]. In another study, naturally occurring flavonoids was noted to possess anti-diabetic effects. As in vitro and animal model's studies demonstrated, they have the ability to prevent diabetes and its complications. The study pointed out that flavonoids improved the pathogenesis of diabetes and its complications through the regulation of glucose metabolism, hepatic enzymes activities, and a lipid profile [38]. An earlier study evaluated the protective effect of tannins from *Ficus racemosa* on the lipid profile and antioxidant parameters in high fat meal and streptozotocin induced hypercholesteremia associated diabetes model in rats. In the study, the crude tannin fraction was separated from the acetone (70% v/v) bark extract of *F. racemosa*. Oral administration of tannin fraction (TF) (100 & 200 mg/kg body weight) to rats fed with high fat meal for 30 days (4% cholesterol, 1% cholic acid, 0.5% egg albumin) and injected with streptozotocin (35 mg/kg i.p. in citrate buffer on 14th day). The researchers recorded that the administration of tannin fractions significantly ($p < 0.05$) reversed the increased blood glucose, total cholesterol, triglycerides, low density lipoprotein and also significantly restored the insulin and high density lipoprotein in the serum. In addition tannins significantly restored the activity of antioxidant enzymes such as superoxide dismutase, catalase and decreased the glutathione peroxidase, and glutathione, thereby restoring the antioxidant status of the organs to almost normal levels. They concluded that two different doses of tannin supplementation had a favorable effect on plasma glucose and lipid profile concentrations; and also had an influence on attenuating oxidative stress in diabetic rats [39]. Additionally, low protein (LP) diet during pregnancy was shown to reduce plasma insulin levels in rodents. The researchers recapped that glucose is the primary insulin secretagogue, and enhanced glucose-stimulated insulin secretion (GSIS) in beta cells contributes to compensation for insulin resistance and maintenance of glucose homeostasis during pregnancy. In the study, the researchers hypothesized that plasma insulin levels in pregnant rats fed LP diet are reduced due to disrupted GSIS of pancreatic islets. They firstly confirmed reduced plasma insulin levels, then investigated in vivo insulin secretion by glucose tolerance test and ex vivo GSIS of pancreatic islets in the presence of glucose at different doses, and KCl, glibenclamide, and L-arginine. The researchers discovered that plasma insulin levels were unaltered on day 10, but significantly reduced on days 14–22 of pregnancy in rats fed LP diet compared to those of control (CT) rats; also, insulin sensitivity was unchanged, but glucose intolerance was more severe in pregnant rats fed LP diet; GSIS in pancreatic islets was lower in LP rats compared to CT rats in the presence of glucose, KCl, and glibenclamide, and the response to L-arginine was abolished in LP rats; and the total insulin content in pancreatic islets and expression of Ins2 were reduced in LP rats, but expression of Gcg was unaltered. These studies demonstrated that decreased GSIS in beta cells of low protein rats contributed to reduced plasma insulin levels [40]. This was an indication that the presence of protein contributed to the hypoglycemic effects of *Grona trifloral*. These were insights to the profound hypoglycemic potentials of phytochemical compounds. The results of the acute toxicity study showed that *Grona trifloral* ethanol leaf extract exhibited high safety profile as no death of mice occurred at the high dose of 5,000 mg/kg body weight. This is in support of the generally acclaimed fact that herbal drugs are safe which resulted to the increase in their use for pharmacological purposes. A certain study clearly recognize that despite the fact that herbal medicines are not completely free from the possibility of toxicity or adverse effects, they are widely considered to be of lower risk compared with synthetic drugs [41]. According to the results of the diabetes study, the percentage decrease in blood glucose levels (%) were well recorded. It was certain that *Grona trifloral* ethanol leaf extract had a profound anti-diabetic potentials that is dose related; which at the highest tested dose of 500 mg/kg body weight became more potent than the standard anti-diabetic drug metformin. This was evident on the day 14th when metformin recorded a percentage decrease in blood glucose level of 74.21% as against the 75.83% obtained for 500 mg/kg body weight of *Grona trifloral* ethanol leaf extract. More so, it is obvious from the result that 500 mg/kg body weight extract had faster effect than metformin because on the 3rd day of treatment, only *Grona trifloral* leaf extract (500 mg/kg body weight) had a significant decrease in blood glucose level ($p < 0.05$) achieving a decrease of 34.55% as compared to the 15.02% noted for metformin. In a particular study done in Ethiopia, diabetes mellitus was recognized as a serious, chronic disease that occurs either when the pancreas does not produce enough insulin, or when the body can't effectively use insulin; and herbal medicines have been commonly used by diabetic patients for the treatment of diabetes mellitus. This study was a review in which findings from different researches related to *in vivo* and invitro anti-diabetic activities of medicinal plants in Ethiopia were searched from different databases, such as Web of Science, Google Scholar, Medline, Scopus, and PubMed, using English key terms. The researchers reported that different medicinal plant

parts were used experimentally for anti-diabetic effects in Ethiopia. Among these, leaves (69%) were the most commonly investigated medicinal plant parts followed by roots (14%) and seeds (7%). Most of the investigations were completed with hydro-methanolic extracts to obtain a higher percentage of yield. Medicinal plants such as *Thymus schemperi* R*, Thymus vulgaris* L*, Hagenia abyssinica, Aloe megalacantha baker, Aloe moticola* Reyonolds, *Aloe pulecherrima* Gilbert & sebseb*, Bersama abyssinica* fresen*, and Rubus Erlangeri* Engl among others were studied and the review gave collective evidence on the potential anti-diabetic activities of medicinal plants in Ethiopia [42]. In another study, diabetes mellitus was highlighted as an insulin-related metabolic disorder characterized by prolonged hyperglycemia which has been primarily treated with various synthetic drugs that ameliorate the altered glycemic status in diabetic subjects. Although synthetic drugs are efficient, they have notable side effects together with their beneficial action. According to the study, medicinal plants have been used since ages to treat diabetes and associated conditions in various healthcare systems around the globe and a vast number of medicinal plants have been attributed to have anti-diabetic potential in preliminary assays. The review gave a general picture of medicinal plants that had been assessed in human diabetic subjects, which can be developed either in combination with other medicinal plants or alone as medication for diabetes thus confirming the efficacy of medicinal plants in the treatment of diabetes [43]. A different study also reiterated that culinary herbs and spices are widely used as a traditional medicine in the treatment of diabetes and its complications, and that there are several scientific studies in the literature supporting the use of these medicinal plants. However, there is often a lack of knowledge on the bioactive compounds of these herbs and spices and their mechanisms of action. The study therefore aimed at using inverse virtual screening to provide insights into the bioactive compounds of common herbs and spices, and their potential molecular mechanisms of action in the treatment of diabetes. The researchers screened a library of over 2300 compounds derived from 30 common herbs and spices in silico with the DIA-DB web server against 18 known diabetes drug targets. Over 900 compounds from the herbs and spices library were observed to have potential anti-diabetic activity and liquorice, hops, fennel, rosemary, and fenugreek were observed to be particularly enriched with potential anti-diabetic compounds. A large percentage of the compounds were observed to be potential polypharmacological agents regulating three or more anti-diabetic drug targets and included compounds such as achillin B from yarrow, asparasaponin I from fenugreek, bisdemethoxycurcumin from turmeric, carlinoside from lemongrass, cinnamtannin B1 from cinnamon, crocin from saffron and glabridin from liquorice. The major targets identified for the herbs and spices compounds were dipeptidyl peptidase-4 (DPP4), intestinal maltase-glucoamylase (MGAM), liver receptor homolog-1 (NR5A2), pancreatic alpha-amylase (AM2A), peroxisome proliferator-activated receptor alpha (PPARA), protein tyrosine phosphatase non-receptor type 9 (PTPN9), and retinol binding protein-4 (RBP4) with over 250 compounds observed to be potential inhibitors of these particular protein targets. Only bay leaves, liquorice and thyme were found to contain compounds that could potentially regulate all 18 protein targets followed by black pepper, cumin, dill, hops and marjoram with 17 protein targets. In most cases more than one compound within a given plant could potentially regulate a particular protein target. The researchers also observed that through this multi-compound-multi target regulation of these specific protein targets that the major anti-diabetic effects of reduced hyperglycemia and hyperlipidemia of the herbs and spices could be explained and they concluded that the anti-diabetic potential of common culinary herbs and spices was the result of the collective action of more than one bioactive compound regulating and restoring several deregulated and interconnected diabetic biological processes [44]. In a more specific study, *Becium grandiflorum* which has been used traditionally for treatment of different ailments including diabetes mellitus was evaluated of its anti-diabetic effects in streptozotocin (STZ)-induced diabetic mice. In the study, hydro-ethanolic (30:70) leaf extract of *Becium grandiflorum* was evaluated in STZ (45 mg/kg body weight)-induced diabetic and normal mice. hypoglycemic, oral glucose tolerance and body weight change effects of the extract were assessed after administering three doses of the extract (200, 400 and 600 mg/kg body weight), glibenclamide 5 mg/kg body weight (reference drug) and 2% Tween 80 (vehicle). All doses of the extract (200 mg/kg ($p < 0.05$), 400 mg/kg ($p < 0.05$) and 600 mg/kg ($p < 0.01$)) and glibenclamide 5 mg/kg ($p < 0.001$) showed statistically significant blood glucose level reduction in normal mice as compared to Tween 80. The hydroalcoholic extract at a dose of 200 mg/kg ($p < 0.05$), 400 mg/kg ($p < 0.01$) and 600 mg/kg ($p < 0.001$) showed better blood glucose tolerance after 60, 120 and 180 minutes treatment duration in normal mice as compared to negative control. In diabetic mice, *Becium grandiflorum* doses and the reference drug caused maximum reduction in blood glucose level at the end of the 15th day of treatment by 17.61%, 22.52%, 24.62% and 34.12%, respectively. The extract's doses and the standard drug showed significant ($p < 0.05$) improvement in body weight while the diabetic control continued to lose their body weight. The researchers concluded that *Becium grandiflorum* exhibits anti-hyperglycemic activity in STZ-induced diabetic mice, and shows improvement in oral glucose tolerance and body weight [45].

5. Conclusion

In conclusion, *Grona trifloral* ethanol leaf extract had dose dependent increase in anti-hyperglycemic effects and at the highest tested dose of 500 mg/kg body weight, *Grona trifloral* hypoglycemic activities surpassed that of metformin (a

standard anti-diabetic drug); with 75.83% and 74.02% reduction of blood glucose levels respectively on the 14th day of treatment.

Compliance with ethical standards

Acknowledgments

I wish to acknowledge Mr Ikeh Chibueze and Mr Joseph Nwarishi, the laboratory technologists whose effort ensured that this work was completed successfully.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Animals were handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals (Pub No. 85-23, revised 1985). Ethical approval for handling of laboratory animals was obtained from the Animal Research and Ethics Committee, Nnamdi Azikiwe University, Awka. The ethical approval number is NAU/AREC/2023/00077.

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