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Effect of *Suregada zanzibariensis* (Baill) root extract on blood sugar levels, lipid profile and pancreatic histology on streptozotocin-nicotinamide induced diabetes mellitus rats

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ABSTRACT

Background & Aim: Ethnobotanical studies have shown that *Suregada zanzibariensis* roots are used by traditional healers and the community for the treatment of diabetes mellitus. Therefore, this study was undertaken to investigate the effect of the ethyl acetate extract of *Suregada zanzibariensis* roots (EAESZ) on blood sugar levels, lipid profile, and pancreatic histology in streptozotocin-nicotinamide-induced diabetic rats.

Experimental: Rats were induced to have diabetes by interstitial injection of streptozotocin nicotinamide, followed by daily oral administration of the ethyl acetate extract of *Suregada zanzibariensis* roots (EAESZ) for 28 days at doses of 350, 500, and 700 mg/kg body weight. The effect of EAESZ on serum lipid profiles and pancreatic β-cells in diabetic rats were examined after 28 days of administration of the extract. While fasting, blood glucose levels were measured every seven days.

Results: The administration of EAESZ at doses of 350, 500, and 750 mg/kg significantly decreased the fasting blood glucose levels compared to the diabetic control rats. Also, total cholesterol (TC), low-density lipoprotein (LDL), and triglyceride (TG) levels decreased while high-density lipoprotein (HDL) levels increased in all treated groups compared to diabetic control rats. Furthermore, there was no significant difference ($P>0.05$) in body weight of treated diabetic rats compared to standard control diabetic rats, while there was a significant difference ($P<0.05$) with diabetic control rats.

Recommended applications/industries: These results indicate that EAESZ has high antidiabetic potential along with significant hypoglycemic and hypolipidemic effects. However, more studies are needed to identify and isolate compounds responsible for those properties.

1. Introduction

Diabetes mellitus (DM) is a common non-communicable disease and metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Gitleman, 2014). Failure of enough insulin production or its action makes the cells of the body also fail to absorb sufficient glucose from the blood; this later causes a rise in blood glucose levels, which is termed hyperglycemia (Jagtap *et al.*, 2021). If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs such as the kidneys, liver, eyes, nerves, heart, and blood vessels. Complications in some of these organs can lead to death (Pari and Venkateswaran, 2003).

DM is one of the world's most challenging public health problems due to its high and growing prevalence (Soumyanath, 2005; Gregg *et al.*, 2021). According to the World Health Organization (WHO, 2021), about 422 million people worldwide have diabetes, the majority living in low- and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. The newsroom of the WHO also reported that the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014. Furthermore, 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 (Risk and Collaboration, 2016; WHO 2021). Medicinal plants have been used as a source of medicine throughout the world, and 80–85% of populations rely on these plants, using the extracts or their active ingredients as traditional medicine to address their basic healthcare needs (Ignacimuthu *et al.*, 2006). Due to accessibility and cultural acceptance, more than 80% of the population in Sub-Saharan Africa uses traditional medicine as their main source of healthcare (Keter and Mutiso, 2012). Numerous phytochemicals have been taken from medicinal plants and utilized as medications. For instance, metformin, an oral hypoglycemic drug, was extracted from the medicinal plant *Galega officinalis* (Li *et al.*, 2004). People in Tanzania use a wide range of natural resources to address their healthcare needs, and it is believed that at least 60% of the population uses traditional medicines (Stanifer *et al.*, 2015). Traditional diabetes treatments include the use of several medicinal plants (Moshi and Mbwambo, 2002). According to Runyoro *et al.* (2006), 77.1% of DM patients in Kilimanjaro and Kilosa use herbal medications to treat their condition (Stanifer *et al.*, 2015). The decoction from the root of *Suregada zanzibariensis* has been utilized in Kilosa to treat DM and other diseases like gonorrhea, stomach aches, chest pain, hernias, pneumonia, and snake bites, while the leaves are used to treat skin infections and ancylostomiasis (Moshi and Mbwambo, 2002; Innocent *et al.*, 2010).

The Suregada genus has about 30 species, 22 of which are located in Africa (Mangisa *et al.*, 2019). *Suregada zanzibariensis* Baill is widely distributed throughout the east coast of Africa, primarily in Tanzania's Ruvuma, Bagamoyo, Zanzibar, and Morogoro (Chhabra *et al.*, 1993; Luke, 2004). On the other hand, it has been reported that traditional healers use the root decoction of *Suregada zanzibariensis* to treat and manage DM (Moshi and Mbwambo, 2002), but this claim has not been scientifically proven. Therefore, the aim of this study is to investigate the antidiabetic activity of *Suregada zanzibariensis* in streptozotocin-induced type 2 diabetic rats by studying the effect on blood sugar levels, lipid profile, and pancreatic histology.

2. Materials and Methods

2.1. Description of the study area and sample collection

On the Mwalimu JK Nyerere Mlimani campus of the University of Dar es Salaam, fresh *S. zanzibariensis* roots weighing 2 kg were taken in January 2022 from a bushy area close to a moist spot. The collection site is situated at a height of 104 meters (GPS: 37m 521958 utm 9250774).

2.2. Plant sampling and preprocessing

A plant taxonomist in the field made an initial determination of the type of plant. Using fresh leaves and roots of *S.zanzibariensis*, the gathered fresh plant roots were validated at the herbarium of the Department of Botany, University of Dar-es-Salaam, where voucher specimens were coded and given the voucher specimen number FMM 4140. Next, fresh roots of the *S.zanzibariensis* plant were gathered for the investigation. Fresh roots were carefully washed in tap water, rinsed in distilled water, and allowed to dry in the shade in a well-ventilated place. Dry samples were macerated after 21 days and kept at room temperature for testing purposes. For extraction, analytical grade ethyl acetate of 99.5% purity, produced by Loba Chemie PVT. LTD. was used. Weighed at 500 g, powdered *S. zanzibariensis* roots were steeped for 1.5 liters at room temperature in ethyl acetate. The mixture was shaken once every 12 hours for two days before being filtered through Whatman No. 1 filter paper, which has an 11 µm pore size. According to Targett (1979) a rotary evaporator with the model number RE-501 made by Labs Nova (USA) was used to dry the ethyl acetate extract at 40 °C. The extract was stored in a dry container and kept at 4 ºC until the day of usage.

2.3. Experimental animals

The treatment and handling of the animals used in this investigation complied with generally accepted

ethical standards for the use of laboratory animals (NRCNA, 2010). Eight-week-old male rats weighing between 85 and 100 g were taken from the labs at Sokoine University of Agriculture (SUA)-Morogoro and nurtured at the zoology lab at the University of Dar es Salaam. According to NRCNA, (2010) and Tafesse *et al.* (2017), the animals were housed in cages with three rats per cage at room temperature with a 12 h/12 h light/dark cycle. The animals were given standard pellets as food and water throughout the trial, with the exception of the fasting period.

2.4. Antidiabetic type 2 assay

2.4.1. Preparation of citrate buffer for dissolving streptozotocin

To prepare 100 cm^3 of citrate buffer at pH 4.5, 24.5 mL sodium citrate (0.1 M), 25.5 mL citric acid (0.1M), and 50 mL distilled water were mixed together. Whereby, aqueous sodium citrate (MW 294 g/mol) was made by dissolving 3.57 g of sodium citrate in 100 mL distilled water, whereas aqueous citric acid (MW 210 g/mol) was prepared by dissolving 2.1 g of citric acid in 100 mL distilled water. The pH of citrate buffer was measured using pH meter HI2211 pH/ORP Meter Hanna instruments

2.4.2. Preparation of phosphate buffer for dissolving nicotinamide

Sodium dihydrogen orthophosphate 0.2 M (14 mL), disodium hydrogen orthophosphate 0.2M (36 mL), and 50 mL distilled water were combined to make 100 mL of phosphate buffer at pH 7.2. Whereby, dissolving 3.12 g sodium dihydrogen orthophosphate (mw 156 g/mol) in 100 mL distilled water yielded 0.2M sodium dihydrogen orthophosphate, while dissolving 2.83 g disodium hydrogen orthophosphate (MW 142 g/mol) in 100 mL distilled water yielded 0.2M disodium hydrogen orthophosphate. The pH of the phosphate buffer was determined using a Hanna pH meter (HI2211 pH/ORP Meter).

2.4.3. Induction of diabetes mellitus type 2

There were six groups of rats. Diabetes was induced in overnight fasted rats according to the method of Jagtap *et al.* (2021). Diabetes was induced in rats by intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg in 0.1 mL citrate buffer (pH 4.5) 15 min after a dose of nicotinamide (NIC) injection (120 mg/kg in 0.1 mL normal saline) in all the groups except for Group I which was non-diabetic control i.e., Normal control. Animals were fed with glucose solution (5%) for 12 h to avoid hypoglycaemia. Hyperglycaemia was confirmed after 3 days. Rats having blood glucose >250 mg/dL were selected for the study.

2.4.4. Evaluation of antidiabetic activity

Six groups of three rats each were formed, Group I served as healthy control rats; Group II had diabetic rats who received water every day for 28 days without any form of treatment. Group III had diabetic rats that were administered the standard drug metformin. (250 mg/kg) (The dose of metformin administered to rats in this study was calculated according to a clinically relevant human dose based on body surface area (Jagtap *et al.*, 2021); Group IV, V and IV of diabetic rats were administered *S. zanzibariensis* extract at doses of 250 mg/kg, 350 mg/kg and 750 mg/kg, respectively, every day for 28 days.

2.4.5. Blood glucose testing

Blood sample was collected by tail vein blood sampling method according to Zou *et al.* (2017). In this method, rats were placed in a plastic restraining holder then the tail was wiped with 70% ethanol, and cleaned with gauze to make the vein clear. A 22 G butterfly needle was inserted into one of the lateral tail veins and blood sample was collected. Fasting glucose level was determine accring to Nagappa *et al.* (2003) on days 1, 7, 14, 21 and 28 using an electronic glucometer (Contour® Plus South Africa). Then, the rats were released from the plastic restraining holder and returned to their cages. During the experimental period, the rats were weighed and the mean changes in body weights were calculated.

2.5. Lipid profiles

The lipid profiling was done according to Jagtap *et al.* (2021) and Sheikh *et al.* (2015). On the 28th day, all animals were euthanized and sacrificed. Blood was collected by cardiac puncture and immediately placed in tubes containing no EDTA and then centrifuged at 4000 r/min at 4℃ for 10 min to obtain serum which was stored at -80ºC. Triglycerides (TGL), low density lipoprotein (LDL), total cholesterol and high-density lipoprotein (HDL) from serum were determined by using Erba XL-100 an auto-analyzer.

2.6 Histopathological analysis

Histopathological analysis was performed following standard laboratory procedure to see if there was effect of roots extract on regeneration of pancreatic β-cells as proposed by Liu *et al.* (2019). Where by, on the 29th day animals were euthanized and sacrificed, then the pancreas was taken for histologic studies. The pancreas was fixed in 10% neutral buffered formalin. The fixed samples were dehydrated in ascending series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5-µ thickness were prepared using a microtome, section was fixed in the slide and stained with hematoxylin and eosin (H+E), and studied under a light microscope. The sections were evaluated based on the severity of the pathological changes.

2.7. Statistical Analysis

The student t test was used in this study to see if there is significant difference between the data mean of treatments and control group. Data was expressed as a mean ± standard deviation; differences among treatments was statistically evaluated using OrginPro version 2019b with the confidence level P<0.05.

3. Results and discussion

3.1 Antidiabetic analysis

3.1.1 Effects of the EAESZ on type II diabetes

The blood glucose level in Group II (Diabetic untreated) was determined to be 316.7± 2.66 mg/dL (Table 1). When compared to Group I and III, Group II's blood glucose level increased significantly (P<0.05). The blood glucose levels in Groups III, IV, V, and VI decreased significantly (P<0.05) during the course of the 28-day treatment plan compared to Group II. In Group VI, slight decrease in blood glucose level at 28 days was observed which was significant (P<0.05) as compared to Group III (Standard control treated with 250 mg/kg, B.W metformin). On the other hand, in Groups V and VI, no significant difference in blood glucose level reduction was observed at 28 days as compared to Group III. The results suggest the potential effects of EAESZ treated at dose of 500 mg/kg at 14, 21 and 28 days of treatment as compared to group III.

A glucosamine nitrosourea molecule known as streptozotocin (STZ) is designated in the IUPAC system as a 2-deoxy-D-glucose derivative of N-methyl-N-nitrosylurea. It only has a 5–15 minute biological half-life (Goyal *et al.*, 2016) and has been extensively utilized to induce diabetes in research animals and in preclinical studies. STZ injection of STZ, leads to STZ accumulation, particularly in pancreatic β-cells through the GLUT2 transporter system, and resulted in β-cell cytotoxicity (Goyal *et al.*, 2016; Jagtap *et al.*, 2021b). Thus, STZ creates diazomethane (DAM), an alkylating chemical that causes DNA methylation and has diabetogenic effects inside of the cells. Additionally, STZ increases the production of diacylglycerol (DAG), Oxygen free radical generation, the flux of glucose through the polyol metabolic pathway, the accumulation of advanced glycation end products, and cytokine secretion, in addition to increasing NADPH levels through either glucose auto-oxidation or DAG production. The development of diabetic rats is the outcome of all of these mechanisms by which STZ damages β-cell DNA hence decrease in body weight (Goyal *et al.*, 2016).

Table 1. Effects of the EAESZ on fasting blood glucose level in STZ-NIC induced diabetic rats. All values are expressed as mean ± Standard Deviation, n = 3.

S/NO	Treatment Group	Fasting Blood Glucose Concentration (mg/dL)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal control	79.46±0.75	79.16±0.49	79.4±1.01	79.2±0.64	79.56±0.92
II	Diabetic untreated	295.1±10.4	310.2±6.60	315.2±3.02	316.03±3.48	316.9±2.66
III	Standard control	303.9±3.02	292.3±3.06	273.7±5.35	238.13±5.89	203.5±5.12
IV	350 mg/kg	305.1±2.01	300±1.35	293.7±4.17	289.3±4.91	280.5±1.32
V	500 mg/kg	302.3±3.55	286.6±7.98	275.8±5.20	253±5.52	237.1±3.51
VI	750 mg/kg	302.3±3.21	289.2±3.70	267.6±6.60	234.1±6.23	213.7±4.90

3.1.2 Effect of EAESZ on body weight of diabetic rats

The body weights of group I (normal non diabetic rats) and group III (diabetic rats treated with metformin rats) were found to be constant. However, body weights drastically dropped over the course of 28 days in group II (Table 2). Over the course of 28 days, rats in group I had considerably larger body weights than

did rats in groups III, IV, V, and VI (P<0.05). Rats in groups III, IV, V, and VI did not significantly differ from one another in terms of body weight (P>0.05).

Table 2. The effect of 28 days of treatment EAESZ on body weight (g) after STZ-NIC induced diabetes in rats. All values are expressed as mean ± Standard Deviation, n = 3.

S/NO	Treatment Group	Fasting Body weight (g)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal control	99.96±1.91	101±3.59	97.5±0.5	100.3±3.51	99.3±1.15
II	Diabetic untreated	100.5±2.48	92.9±4.12	85.3±4.91	72.03±3.44	63.1±5.76
III	Standard control	100.66±1.2	100±1.76	97.5±0.5	94.6±1.36	92.3±2.61
IV	350 mg/kg	103.96±3.9	99.4±3.13	95.5±4.84	93.86±4.0	91.9±2.95
V	500 mg/kg	99.76±2.42	97.6±0.7	95.46±2.8	91.8±3.53	91.5±2.15
VI	750 mg/kg	99.03±0.87	96.63±1.2	91.4±1.02	87.96±2.49	87.53±5.92

In this study, it was discovered that the body weights of the rats in groups I (normal control rats) and group III (diabetes control rats) were stable. Over the course of the next 28 days, the body weights of group II, however, significantly decreased. The STZ-NIC injection, which causes type II diabetes but not treated with metformin or EAESZ, is what caused the rats in group II to lose weight (Nagappa *et al.*, 2003); in contrast, the rats in group I did not have diabetes. When compared to group I, a slight decrease in body weight was also seen in group III that had been given metformin treatment; this could have been brought on by the drug's effects, which included treating insulin resistance and promoting β-cell regeneration (Jagtap *et al.*, 2021; Zhao *et al.*, 2020). Furthermore, the body weights of the rats in groups IV, V, and VI that were given EAESZ at doses of 350 mg/kg, 500 mg/kg, and 750 mg/kg, respectively, did not substantially differ from one another (P<0.05). This may be caused by the presence of antihyperglycemic substances including phenol, 3,5-bis (1,1-dimethylethyl),and squalene (Spanova and Daum 2011). These compounds are known to aid in β-cell regeneration and boost cell insulin sensitivity (Nazarudin *et al.*, 2020; Zhao *et al.*, 2020; Spanova and Daum, 2011).

3.2 Effects of the EAESZ on lipid profile of diabetic rats

In Group III rats (treated with metformin) and Groups IV, V, and VI (treated with EAESZ at doses of 350 mg/kg, 500 mg/kg, and 750 mg/kg, respectively) serum tryglycerides, serum cholesterol, and serum LDL were significantly reduced for 28 days, while HDL was significantly raised compared to Group I (P<0.05). Additionally, there was a substantial increase in serum tryglycerides, serum cholesterol, and serum LDL while a significant reduction in serum HDL was observed in group II (the diabetic untreated) as compared to groups I and III (P>0.05). Additionally, group III, IV, V, and VI's blood tryglycerides, cholesterol, serum LDL, and serum HDL did not significantly differ from one another as shown in Figure 1.

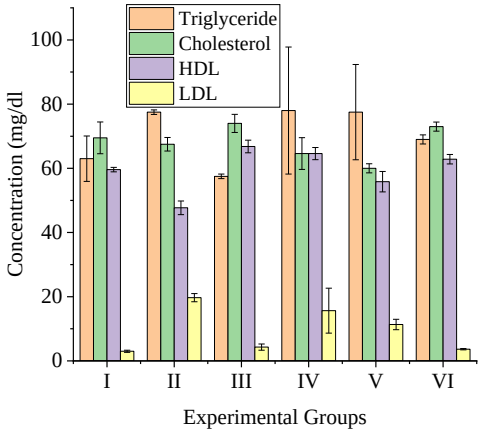

Figure 1. Effect of EAESZ on lipid profile in streptozotocin nicotinamide -induced diabetic rats after 28 days of treatment. Each point represents a mean ± standard deviation and n = 3

Increased levels of lipolysis are caused by insulin resistance (type II diabetes), and an increase in insulin sensitivity prevents excessive lipolysis in adipose tissue (Zhao *et al.*, 2020). The effects of the drug metformin, which was used for therapy for 28 days, were responsible for the decrease in serum tryglycerides, serum cholesterol, serum LDL, and increase in serum HDL in diabetic-induced rats in group III (Standard control). Since metformin is used to treat insulin resistance, it prevents adipocytes from undergoing induced lipolysis (Jagtap *et al.*, 2021b; Zhao *et al.*, 2020). In the current study, groups IV, V, and VI all experienced increases in serum HDL and decreases in serum tryglycerides, cholesterol, and LDL. This might

be because hypoglycemic substances like phenol, 3,5-bis (1,1-dimethylethyl), found in EAESZ are known to promote insulin sensitivity and inhibit excessive lipolysis in adipose tissue (Nazarudin *et al.*, 2020; Zhao *et al.*, 2020). Additionally, it might be brought on by the presence of squalene in EAESZ (input reference of toxicity paper), which has chemopreventive and hypocholesterolemic properties, lowering serum cholesterol and LDL levels (Spanova and Daum, 2011).

3.3 Effect of EAESZ on pancreatic cells

One Langerhans islets from untreated and normal diabetic rats are shown in Figures 2 and 3, respectively. Compared to these two figures, it is obvious that diabetic rats have fewer pancreatic islets and beta cells. However, histophatological analysis of the pancreas of treated diabetic rats at various doses (350, 500, and 750 mg/kg) revealed an increase in the number of pancreatic islets and the number of β-cells, as well as a decrease in the number of activated lymphocytes and macrophages, compared to the untreated diabetic rats (Figures 5, 6 and 7, respectively). Additionally, diabetic rats treated with the standard medication that is Metformin (Figure 4) had more pancreatic islets and β-cells than untreated diabetic rats, whereas diabetic rats treated with EAESZ at doses of 500 mg/kg and 750 mg/kg did not vary from diabetic rats treated with standard drug.

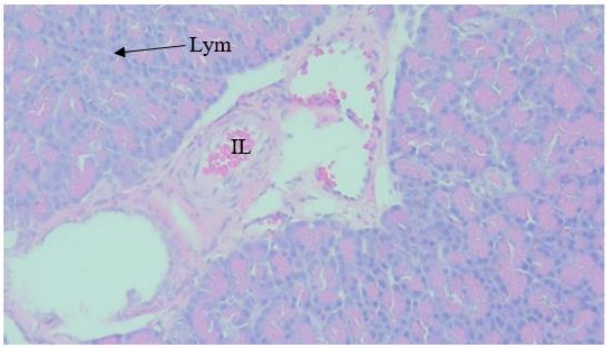

Figure 2. From the pathological examination of diabetic untreated rats, a large number of lymphocytes (Lym) are present across the area, the irregular shape of the islet of Langerhans (IL), with no β-cells at the central. indicating tissue injury (40x hematoxylin-eosin).

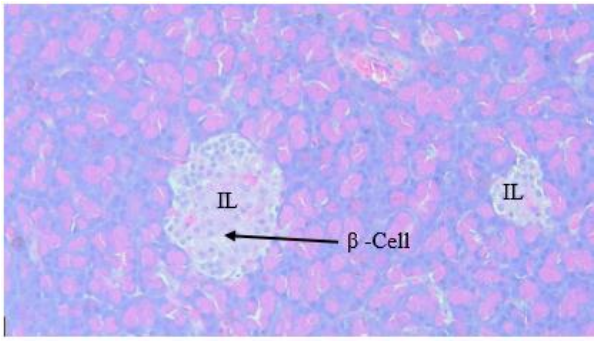

Figure 3. Two perfect islets of Langerhans (IL) with numerous β-cells at the center, a consistent oval shape, and a distinct boundary between them and surrounding acinar cells (peri islet acinar and tele islet acinar) are visible from the pathological examination of a normal rat given standard food and water for 28 days, indicating no tissue injury (40x hematoxylin-eosin).

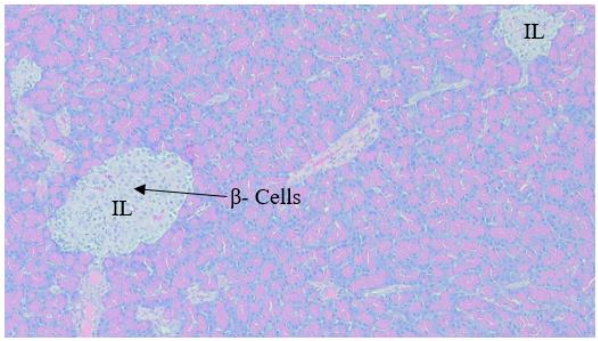

Figure 4. From the pathological examination of diabetic rats treated with metformin at doses of 250 mg/kg for 28 days, two ovoid-shaped islets of Langerhans (IL) with β-cells at the central and clear boundary with the surrounding acinar cells, the inflammatory infiltrate has vanished (hematoxylin and eosin, 40x).

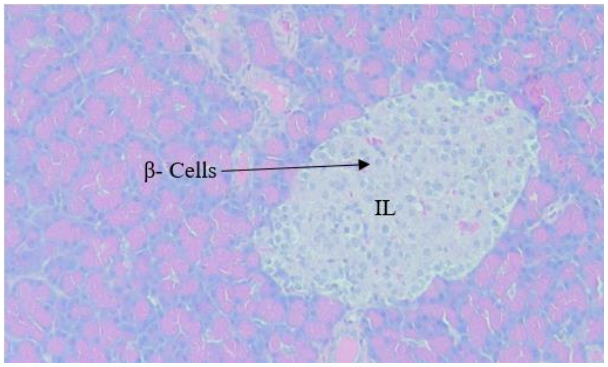

Figure 5. One perfect islets of Langerhans (IL) with numerous β-cells at the center, a consistent oval shape, and a distinct boundary between them and surrounding acinar cells (peri islet acinar and tele islet acinar) are visible from the pathological examination of a normal rat given 350 mg/kg dosages of EAESZ for 28 days, indicating no tissue injury (40x hematoxylin-eosin).

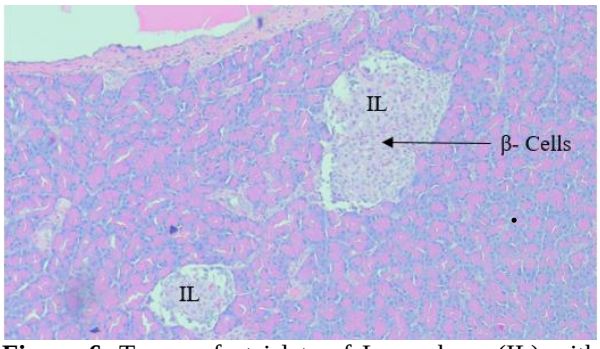

Figure 6. Two perfect islets of Langerhans (IL) with numerous β-cells at the center, a consistent oval shape, and a distinct boundary between them and surrounding acinar cells (peri islet acinar and tele islet acinar) are visible from the pathological examination of a normal rat given 500 mg/kg dosages of EAESZ for 28 days, indicating no tissue injury (40x hematoxylin-eosin).

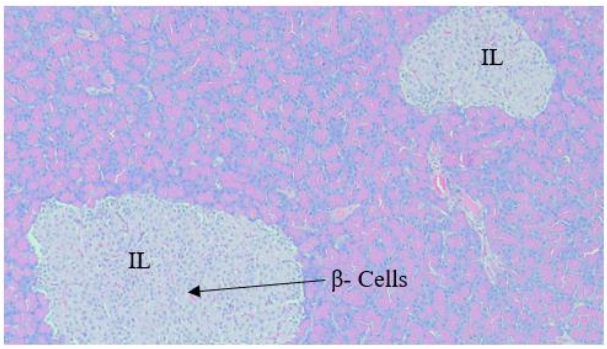

Figure 7. Two perfect islets of Langerhans (IL) with numerous β-cells at the center, a consistent oval shape, and a distinct boundary between them and surrounding acinar cells (peri islet acinar and tele islet acinar) are visible from the pathological examination of a normal rat given 750 mg/kg dosages of EAESZ for 28 days, indicating no tissue injury (40x hematoxylin-eosin).

However, histophathological analysis of the pancreas of the treated diabetic rats at various doses (350, 500, and 750 mg/kg) revealed an increase in the number of pancreatic islets and the number of β-cells, as well as a decrease in the number of activated lymphocytes and macrophages, compared to the untreated diabetic rats (respectively). Additionally, diabetic rats treated with the standard medication that is Metformin (Figure 4) had more pancreatic islets and β-cells than untreated diabetic rats, whereas diabetic rats treated with EAESZ at doses of 500 mg/kg and 750 mg/kg did not vary from diabetic rats treated with standard drug. Furthermore, the islets are atrophic, tiny, and have an uneven shape, as seen in Figure 2. The majority of the islets' cells are tiny, degranulated and black. In the β-cells of some islets, there is severe vacuolation and degranulation. The islets also show signs of inflammation, or "insulitis" (Figure 2). Within and around the afflicted islets, an exudate that is predominantly composed of lymphocytes with a small number of macrophages and neutrophils is visible.

Therefore, the fact that diabetic rats treated with EAESZ had an increase in both the number of pancreatic islets and β-cells suggests that the extract may contain elements that promote the regeneration of β-cells. This is because the pancreas contains cells that are dormant but have the ability to regenerate when triggered (Jagtap *et al.*, 2021; Monfared *et al.*, 2006). As a result, the remaining cells can multiply to replace the missing ones. Results of lipid profiling, fasting blood glucose, and histological studies all point to the possibility of islet regeneration following plant extract therapy.

4. Conclusion

In the current study, streptozotocin nicotinamide-induced diabetic rats received EAESZ administration, and this treatment significantly reduced blood sugar levels. EAESZ also improve the lipid profile, which includes total cholesterol, triacylglycerol, high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein. The study may support a traditional healer's use of *S. zanzibariensis* roots for the treatment of diabetes mellitus based on the observed antidiabetic activity of EAESZ. Based on the results of the current study, larger-scale pharmacological studies are required to confirm the precise compounds found in *Suregada zanzibariensis* (BAIL that are responsible for health benefits like hypocholesterolemic and antihyperglycemic effects. Additionally, more study is needed to determine exactly how the plant extract promotes islet regeneration.

5. Acknowledgment

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