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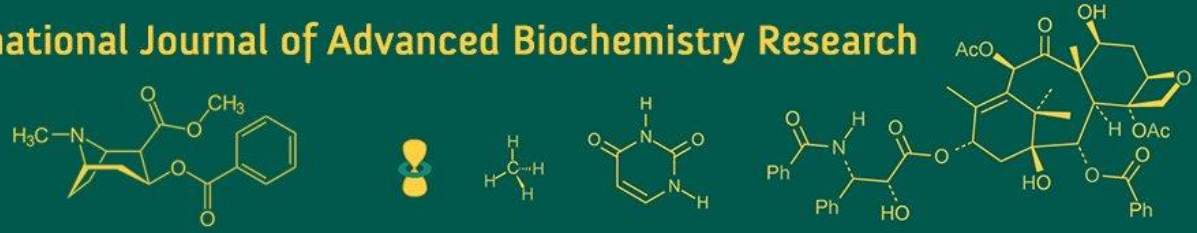

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Effect of ethanol extract of *Andrographis paniculata* leaves on selected biochemical parameters in alloxan-induced hyperglycaemic wistar albino rats

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Abstract
Using alloxan-induced hyperglycaemic Wistar albino rats, this research analysed the impact of ethanol extract of *Andrographis paniculata* leaves on selected biochemical markers. Thirty (30) male rats weighing between 200-250 g were divided into six (6) groups comprising of; normal control (food and water only), positive control (0.1 mg/kg BW of Glimepiride), negative control (untreated), treated (500 mg/kg, 1000 mg/kg and 1500 mg/kg BW of extract) labelled groups 1-6 respectively. Following 21 days of once-daily oral administration, the animals were sacrificed, and biochemical and histological analyses were performed on samples of blood and pancreas. Results revealed a significant decrease ($p \leq 0.05$) in plasma concentrations of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) in groups 4-6; a non-significant decrease ($p \geq 0.05$) in plasma concentrations of high density lipoprotein cholesterol (HDL-C) in groups 4-6; a significant increase ($p \leq 0.05$) in percentage inhibition potency of plasma α-glucosidase enzyme activity in groups 4-6 and a significant increase ($p \leq 0.05$) in plasma concentrations of insulin in groups 4-6 when compared to the control group. A significant decrease ($p \leq 0.05$) in fasting blood glucose level in groups 4-6 and a significant increase ($p \leq 0.05$) in plasma concentrations of insulin in group 4 when compared to the negative control group were observed. Histology of the pancreas (plates 4.1 to 4.6) showed well delineated islets of Langerhans, congested vessels in the connective tissue and normal acini with no intraluminal secretion. This study shows that the *Andrographis paniculata* ethanol leaf extract had a hypoglycaemic effect on alloxan-induced hyperglycaemic Wistar albino rats.

Keywords: *Andrographis paniculata,* hyperglycaemic, α-glucosidase, total cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, high density lipoprotein cholesterol, histology, islets of langerhans

Introduction
Humans have used plants as medicine from the beginning. Despite the fact that their molecular components are often misunderstood, observations on the usage and performance of medicinal plants greatly contribute to the revelation of their therapeutic capabilities such that they are regularly prescribed (Maciel *et al*., 2002; Emerenciano *et al.*, 2019) [38, 21]. The use of medicinal plants has been done since ancient times and may even be considered the origin of modern medicine. Compounds of plant origin have been and still are an important source of compounds for drugs (Salmerón-Manzano *et al.*, 2020) [49]. About 80% of the human population uses only plants found in nature for medical purposes (Hashim *et al*., 2013) [26]. Diabetes, often known as diabetes mellitus, is a metabolic condition caused by insulin insufficiency and persistent hyperglycaemia (high blood sugar). A rise in blood sugar levels may be caused by insufficient or absent insulin synthesis in the pancreas, which normally regulates the body's consumption and storage of glucose. Consequently, this causes problems with how the body metabolises carbohydrates, proteins, and lipids (Smeltzer and Bare, 2010) [51]. The overall incidence of diabetes in Nigeria is 5.7%, or roughly 5 million persons (Uloko *et al*., 2018) [56]. Uloko *et al*. (2018) [56] estimated that almost two-thirds of diabetes cases in Nigeria have not yet been diagnosed, thus this is likely only the beginning. Due to the prevalence of untreated, long-term hyperglycaemia, many people with diabetes already have well-established chronic problems at the time of diagnosis (Olamoyegun *et al*., 2015) [44]. Worldwide, diabetes is estimated to afflict over 460 million individuals, making it

the most common metabolic illness. 34.2 million Americans, or 10.5% of the population, have diabetes in the United States (Dhatariya *et al.,* 2020) [18]. In the conventional medical community, hyperglycaemic (diabetes) condition is treated with a wide variety of therapeutic medications (Andrews and Boyle, 2008) [6]. Although these drugs yield curative results but also produce several detrimental side effects that might require additional clinical attention (Andrews and Boyle, 2008) [6]. It is possible that the diabetic patient may not take their medicine as prescribed due of the unpleasant side effects. It's possible that marital strife might result from these adverse effects (Andrews and Boyle, 2008) [6]. These detrimental side effects include ischemia, hypoglycaemia, allergic reactions, nausea and vomiting, diarrhoea, sexual dysfunction, haemoglobin disorders, lipodystrophy, hypotension, chest pain, loss of appetite, etc. The side effects of orthodox therapies against diabetes have prompted investigation into plant remedy for the disease. The annual herbaceous plant *Andrographis paniculata*, often known as creat or green chiretta, is a member of the family Acanthaceae and is endemic to India and Sri Lanka. Extremely bitter, this plant has been used for generations as a medicinal remedy in Asia, where it has earned the title "King of Bitters" (A-Zherbs, 2006) [9]. The plant, according to Ameh *et al*. (2010) [5], has ovate, pinnate, or lanceolate leaves of varying diameters and grows upright to a height of more than a meter in damp shaded environments. The blooms' little white petals are spotted with purple. The diameter of the dark green stem may be anywhere from 2 mm to 6 mm. Flowers develop into egg-shaped capsules that release a plethora of tiny brown seeds. The seeds of this plant may be found all across the tropical regions of Asia. Eka *et al.*, (2020) [20] reported that the plant could also be obtained at vegetable gardens and vegetations in Akwa Ibom state, Nigeria. A study conducted by Nagajothi *et al*. (2018) [42] investigated the photochemical composition of leaves and stem of *Andrographis paniculata*. They examined both aqueous and ethanol extracts and discovered a wide range of chemical compounds in both extracts, including alkaloids, phenols, tannins, phlobatannins, hydrolyzable tannins, flavonoids, terpenoids and saponins. Interestingly while the ethanol extract did not pass the phlobatannins test it did exhibit cardiac glycosides. The aqueous extract showed higher levels of tannins, terpenoids and saponins when compared to the ethanol extract which had higher levels of alkaloids, total phenols and flavonoids. Ameh *et al* (2010) [5] reported that the LD_{50} of *A. paniculata* in male mice is 11.46 g/kg. Luksamee *et al.* (2019) [37] reported that no acute toxicity were seen in mice when a 5000 mg/kg BW fixed-dose maximum of a standardized extract of *A. paniculata*'s first true leaf (FTLEE) was given orally. Extracts of *A. paniculata* have been subject to various researches on its pharmacological activities which include antimicrobial (Eka *et al*., 2020) [20], hepatoprotective (Chao and Lin, 2012; Abiodun *et al*., 2022) [16, 2], antioxidant (Rafat *et al*., 2010) [48], anti-pyretic (Mahadeva *et al.,* 2000; Kannan *et al.* 2011) [39, 30] and anti-cancer (Hung *et al*., 2010; Liu *et al.,* 2011; Chen *et al*., 2013; Wei *et al*., 2013) [27, 36, 17, 57].

Materials and methods

Animals: Thirty (30) male Wistar albino rats weighing between 200 and 250 grams were purchased from the animal breeding facility of the Department of Biochemistry at the University of Port Harcourt, brought to the Biology laboratory at Vessel of Honour International School located at No 2 Mike Okiro Lane, Road 24 Agip Estate, Port-Harcourt, Rivers State and maintained on a standard diet of rat chow and water.

Plant collection: *Andrographis paniculata* was collected from its natural habitat in Nigeria's Akwa Ibom State; specifically, the community of Ifuho in the Ikot Ekpene Local Government Area. Dr. Chimezie Ekeke of the University of Port Harcourt Herbarium, Department of Plant Science and Biotechnology, identified and authenticated this plant. The voucher number for this specimen is UPH/P/378.

Extraction: *Andrographis paniculata* leaves were freshly harvested from the plant's aerial portions, which were then rinsed with running water. The plant's tops were dried in the shade for two weeks before they were crushed into a coarse powder in a blender. At room temperature for 72 hours, 750 grams (g) of the powdered material was macerated in 1500 milliliter (ml) of 70% (v/v) ethanol. Using a rotary evaporator and a water bath set to 50^{o}C, the filtrate was evaporated to dryness and 120 g of extract was stored in the refrigerator in an airtight container.

Antidiabetic Activity: After acclimatization for seven days, animals used were weighed and their baseline blood glucose concentrations were measured using an ACCU-CHEK Active Glucometer (Code – 333). The normal control group 1 (five rats) was separated and diabetes mellitus was induced in the remaining animals (25) with a single intraperitoneal (i.p) injection of alloxan monohydrate at a dose of 170 mg/kg body weight. Three hours after alloxan administration, the test animals were kept on 50% oral glucose solution to prevent hypoglycaemia usually caused by hyperactivity of the pancreas induced by alloxan two to three hours after its administration. Three days after alloxan injection, the animals were weighed again and their blood glucose concentrations recorded using the same glucometer. All 25 rats (having blood glucose concentrations of 250.0 mg/dl and above) were divided into five groups (2, 3, 4, 5 and 6) of five rats each according to their body weights. Group 2 acted as the positive control and was treated with the reference drug glimepiride at a dose of 0.1 mg/kg body weight while group 3 acted as the negative control and was left untreated. Groups 4, 5 and 6 were treated with the extract of *Andrographis paniculata* at doses of 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weights respectively. All the extracts and drug were dissolved in normal saline and given orally for a period of 21 days. 24 hours after the last treatment, the animals' blood glucose concentrations were measured using the same glucometer. The animals were then anaesthetized with chloroform and sacrificed. Blood and pancreatic tissue samples were then collected for biochemical and histological assays.

Biochemical Assays

Weight: The weights (g) of the Wistar albino rats were determined using an analytical weighing balance

Fasting Blood Glucose (F.B.G): The fasting blood of the Wistar albino rats were determined using an ACCU-CHEK Active Glucometer device and strip. Using a cotton wool dipped in methylated spirit or ethanol absolute, the tail ends

of the rats were cleaned and disinfected to prevent infection. The blood glucose meter and strip were then prepped for action. This was done by inserting the strip correctly in its slide on the glucometer. The blinking of a red dot on the test area indicates it was correctly placed. A tiny abrasive cut was then made to the tail ends of the rats using a sharp razor blade to release few drops of blood which was placed on the strip test area. After a few seconds, the meter gave a value measured in milligram per deciliter (mg/dl). After each test, the tail ends of the rats were disinfected and the used test strip discarded. To convert the value given in mg/dl to millimole per liter (mmol/l), we divide the value by eighteen (18).

Total Cholesterol (TC): The enzymatic endpoint method as described by Allain *et al.,* (1974) was employed. The reagent blank, samples, and standard were put into three separate test tubes and labeled accordingly. The test tubes included portions of distilled water, the standard, and the sample in that order. After adding 500µl of reagent to each of the three labeled test tubes, they were shaken and placed in a water bath at 25 °C to incubate. Within 60 seconds, spectrophotometer measurements at a wavelength of 500 nm were collected of the absorbance of the sample and the standard against the reagent blank.

Calculation

$$\text{Total Cholesterol Conc. (mmol/l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard}$$

Triglyceride (TG) and High Density Lipoprotein Cholesterol (HDL-C): The colorimetric method as described by Tietz (1987) was employed for both assays. The reagent blank, samples, and standard were put into three separate test tubes and labeled accordingly. The test tubes included portions of distilled water, the standard, and the sample in that order. The three labeled test tubes were each given 500 µl (TG) and 1000 µl (HDL-C) of reagent, stirred, and placed in a water bath at 25 degrees Celsius for incubation. Within 60 seconds, spectrophotometer measurements at 500 nm wavelength were collected of the absorbance of the sample and the standard against the reagent blank.

Calculation

$$\text{TG Conc. / HDL-C Conc. (mmol/l)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times \text{Conc. of standard}$$

Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C): The estimation formula methods as described by Friedewald *et al.,* (1972) were employed. LDL-cholesterol was calculated via the formula as shown below:

Conc. of LDL-C (mmol/l) = TC – (HDL-C + VLDL-C)

VLDL-cholesterol was calculated using the formula as shown below:

Conc. of VLDL-C (mmol/l) = Triglycerides/5

Insulin: The solid phase sandwich ELISA technique was employed for the assay. The recommended time for reagents to reach room temperature is 10 minutes. Before using, we gently combined all of the reagents. The next step involved the inserting of the required quantity of coated strips into the container. Equal volumes of insulin standards, control sera, and patient sera were each pipetted into their own wells. We then filled each well with 100 µl of the insulin conjugate reagent. For a full 20 seconds, the ingredients were stirred well together and left out for 60 minutes at ambient temperature (20-25 °C). The fluid was removed from each of the wells which were then Tri-washed with 300 µl of 1X wash buffer and dried with paper towels. 100 µl of TMB substrate was placed into each well and set aside for 15 minutes to incubate at room temperature. We then put 50 µl of the stop solution into each of the wells and shook the plate gently to combine the contents. After 15 minutes, absorbance was measured at 450nm wavelength using an ELISA Reader.

Alpha-glucosidase: The enzyme activity method as described by Abnova was employed. The assay was performed at room temperature of 25 °C. Two wells of a 96-well plate had 20 µL of distilled water (H_2O) added to them. To make a total of 220 µL, we filled one of the wells with 200 µL of H_2O and the other with 200 µL of Calibrator. We moved the 20 µL samples to the other containers. Only the sample wells received the 200 µL Working Reagent. A total of 220 µL of reaction volume was achieved in the sample wells. The plate was gently tapped to combine the ingredients. The plates were read at t = 0 for OD405nm and again at t = 20 for OD405nm + 20 minutes.

Calculation of Results

α-Glucosidase activity of the sample (U/L) is

$$\text{α-Glucosidase Activity} = \frac{OD_{20} - OD_0}{OD_{CALIBRATOR} - OD_{H2O}} \times 250 \text{ (U/L}$$

OD_{20} and OD_0 are OD_{405nm} values of sample at 20 and 0 min, respectively. $OD_{CALIBRATOR}$ and OD_{H2O} are OD_{405nm} values of Calibrator and H_2O at 20 min.

The following formula was used to determine the percentage inhibition.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of test sample}}{\text{Absorbance of Control}} \times 100$$

Histology

Following the procedure outlined by Windsor (1994), the tissues were collected soon after the sacrifice to prevent post-mortem deterioration. The following histological techniques were followed: fixation, dehydration, clearing, embedding, sectioning, preparation and mounting sections on slides, clearing, staining and permanent mounting of sections

Methods of data analysis

The SPSS 20.0 (IBM, United States) statistics software for the social sciences was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to examine the data and Least Significant Difference (LSD) for Post Hoc test of multiple comparisons at $p \leq 0.05$ was used to

identify statistically significant differences. Values were reported as mean±standard error of mean (M±SEM).

Results and Discussion

Table 1: Effect of oral administration of ethanol extract of *Andrographis paniculata* leaves on the body weight of alloxan-induced hyperglycaemic Wistar albino rats

Groups	Weight (B.I) [g]	Weight (A.I) [g]	Weight (A.T) [g]
Normal control (Food and water only)	222.00±2.27^{a}	209.50±3.23^{a}	204.25±2.95^{a}
Positive control (0.1 mg/kg BW of Glimepiride)	229.75±2.95^{a,b}	211.50±2.99	201.25±1.55^{b}
Negative control (untreated)	240.50±2.10^{a,b,c}	220.25±2.14^{a,c}	209.50±3.69
Treated (500 mg/kg BW of extract)	218.75±1.89^{b,c}	203.50±2.40^{c}	209.00±3.49
Treated (1000 mg/kg BW of extract)	236.25±1.93^{a}	209.00±5.12^{c}	209.75±1.03
Treated (1500 mg/kg BW of extract)	226.00±2.97^{c}	205.00±2.55^{c}	214.00±4.20^{a,b}

Key: A.I – After Inducement, A.T – After Treatment, B.I – Before Inducement

Table 2: Effect of oral administration of ethanol extract of *Andrographis paniculata* leaves on the Fasting blood glucose levels of alloxan-induced hyperglycaemic Wistar albino rats

Groups	F.B.G (B.I) [mmol/l]	F.B.G (A.I) [mmol/l]	F.B.G (A.T) [mmol/l]
Normal control (Food and water only)	4.90±0.38^{a}	5.25±0.55^{a}	5.58±0.18^{a}
Positive control (0.1 mg/kg BW of Glimepiride)	4.60±0.26^{b}	22.10±3.57^{a}	11.73±1.86^{a,b}
Negative control (untreated)	6.10±0.47^{a,b,c}	22.05±2.01^{a}	24.15±2.76^{a,b,c}
Treated (500 mg/kg BW of extract)	4.98±0.46	22.75±4.46^{a}	8.78±1.23^{c}
Treated (1000 mg/kg BW of extract)	5.38±0.37	24.45±2.97^{a}	8.95±0.42^{c}
Treated (1500 mg/kg BW of extract)	3.93±0.41^{c}	25.90±2.47^{a}	10.35±1.03^{a,c}

Key: A.I – After Inducement, A.T – After Treatment, B.I – Before Inducement

Table 3: Effect of oral administration of ethanol extract of *Andrographis paniculata* leaves on the plasma lipid profile of alloxan-induced hyperglycaemic Wistar albino rats

	TC (mmol/l)	TG (mmol/l)	HDL-C(mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)
Normal control (Food and water only)	4.30±0.25^{a}	1.64±0.06^{a}	1.55±0.09	3.50±0.20^{a}	0.75±0.03^{a}
Positive control (0.1 mg/kg BW of Glimepiride)	3.00±0.30^{a}	1.23±0.09^{a,b}	1.53±0.09	2.03±0.25^{a}	0.56±0.04^{a,b}
Negative control (untreated)	2.98±0.38^{a}	1.14±0.17^{a}	1.29±0.19	2.26±0.36^{a}	0.53±0.07^{a}
Treated (500 mg/kg BW of extract)	2.80±0.21^{a}	1.00±0.09^{a}	1.48±0.10	1.77±0.20^{a}	0.45±0.04^{a}
Treated (1000 mg/kg BW of extract)	2.70±0.30^{a}	0.89±0.04^{a,b}	1.32±0.07	1.79±0.26^{a}	0.41±0.02^{a,b}
Treated (1500 mg/kg BW of extract)	3.15±0.43^{a}	0.97±0.10^{a}	1.32±0.10	2.28±0.49^{a}	0.45±0.04^{a}

Table 4: Effect of oral administration of ethanol extract of *Andrographis paniculata* leaves on the plasma insulin levels of alloxan-induced hyperglycaemic Wistar albino rats

Groups	Insulin (µIU/ml)
Normal control (Food and water only)	0.38±0.05^{a}
Positive control (0.1 mg/kg BW of Glimepiride)	0.69±0.02^{a}
Negative control (untreated)	0.58±0.07^{c}
Treated (500 mg/kg BW of extract)	0.90±0.03^{a,c}
Treated (1000 mg/kg BW of extract)	0.79±0.11^{a}
Treated (1500 mg/kg BW of extract)	0.79±0.10^{a}

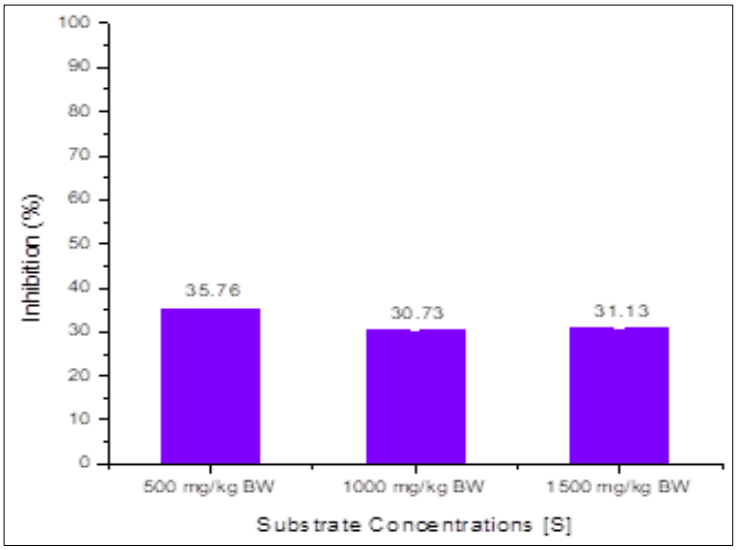

Fig 1: Percentage inhibitory potency of ethanol extracts of *Andrographis paniculata* leaves on α-glucosidase activity of alloxan-induced hyperglycaemic Wistar albino rats

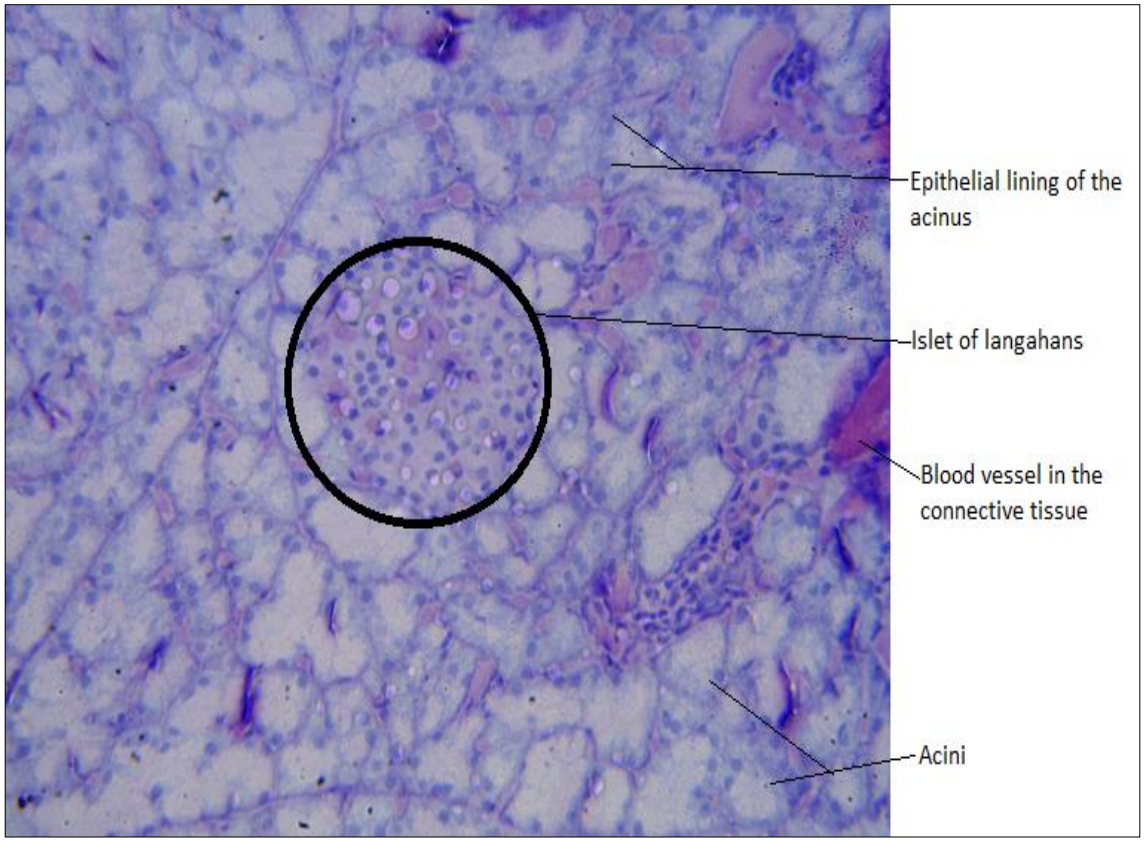

Plate 1: Photomicrograph of pancreatic tissue of normal Wistar albino rat given food and water only, H & E, X400. Plate shows normal pancreatic islet of Langerhans, acini, blood vessels in connective tissue and epithelial lining of the acinus

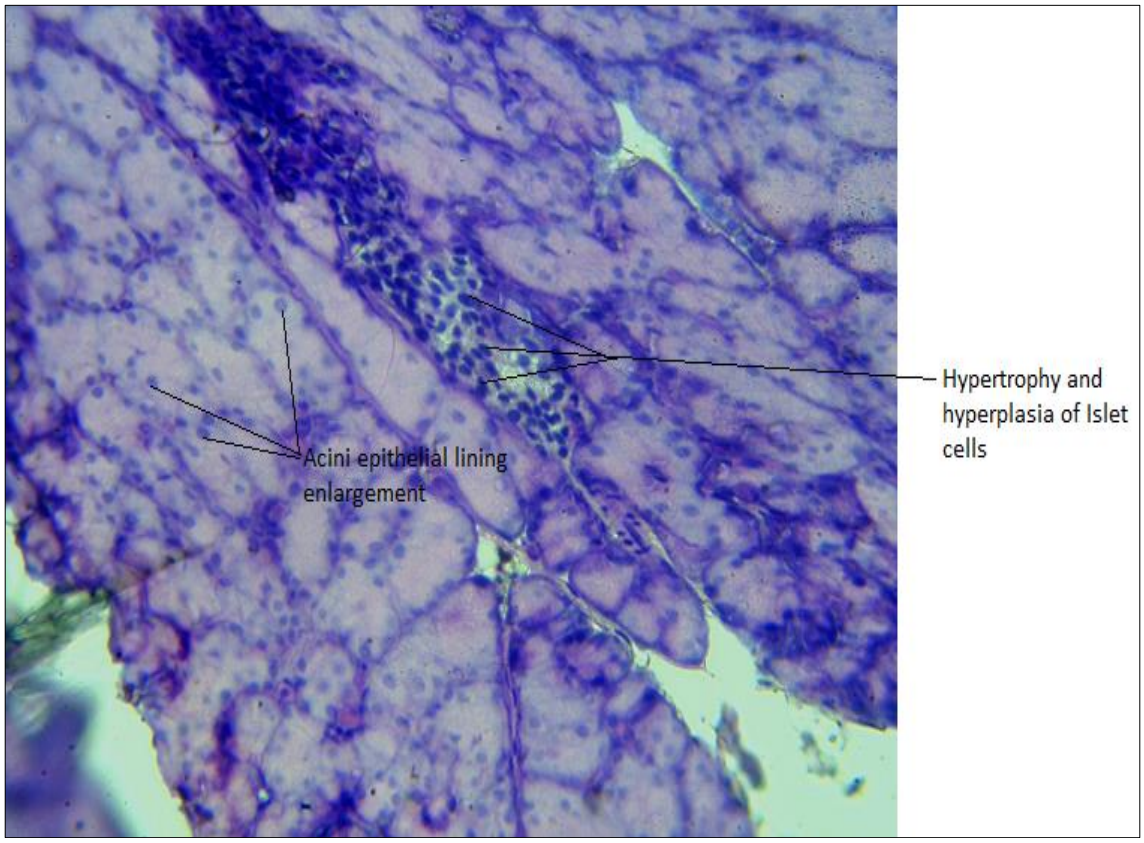

Plate 2: Photomicrograph of pancreatic tissue of hyperglycaemic Wistar albino rat treated with 0.1 mg/kg BW of Glimepiride, H & E, X400. Plate shows hypertrophy and hyperplasia of Islet of Langerhans cells, an indication of mild distortion. The acini epithelial lining cells also show enlargement

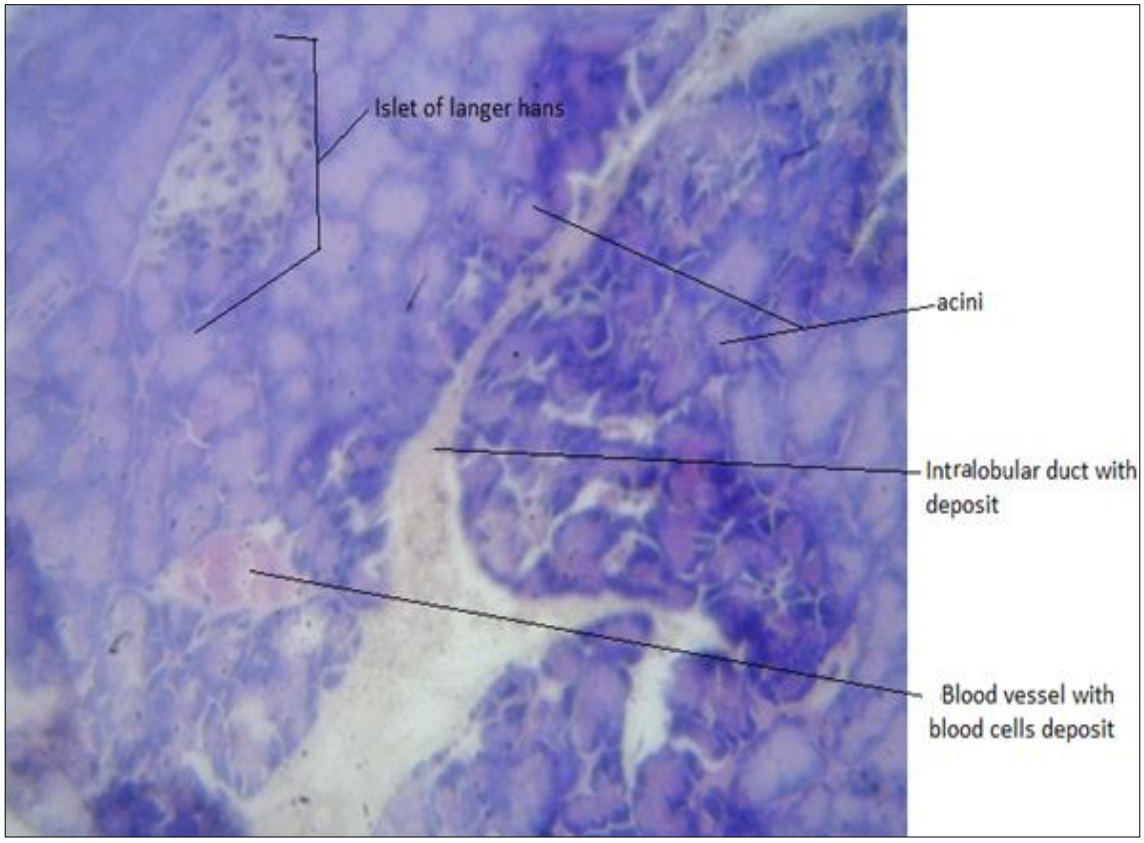

Plate 4.3: Photomicrograph of pancreatic tissue of hyperglycaemic Wistar albino rat left untreated, H & E, X400. Plate shows congestion of blood vessel, Interlobular duct congestion and acini with intraluminal secretion. There is degeneration (pygnosis) of Islet of Langerhans cells with edematous appearance. Tissue distortion is indicated

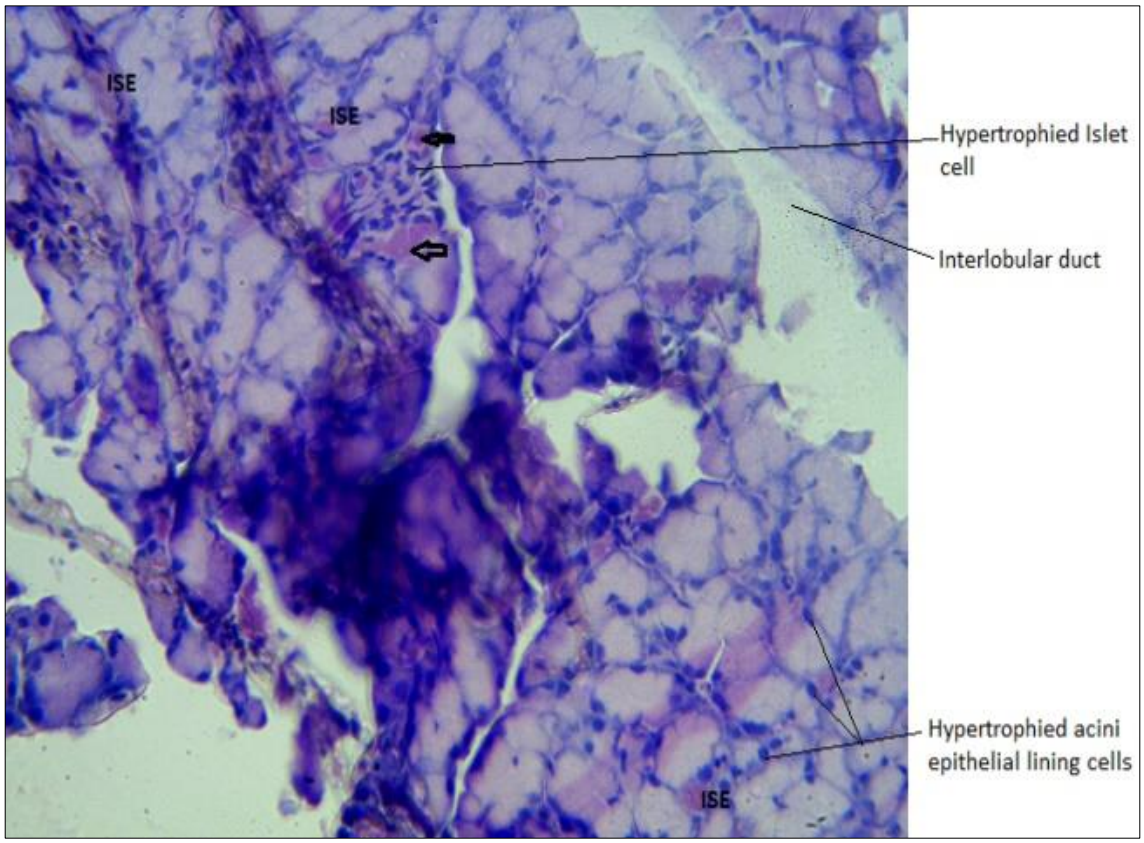

Plate 4: Photomicrograph of pancreatic tissue of hyperglycaemic Wistar albino rat treated with 500 mg/kg BW of extract, H & E, x400. Plate shows tissue with hypertrophied islet cells with congestion (edema- arrowed). The epithelial cells lining the acini show notable hypertrophy. There is also indication of interstitial space edema (ISE). Tissue distortion is indicated

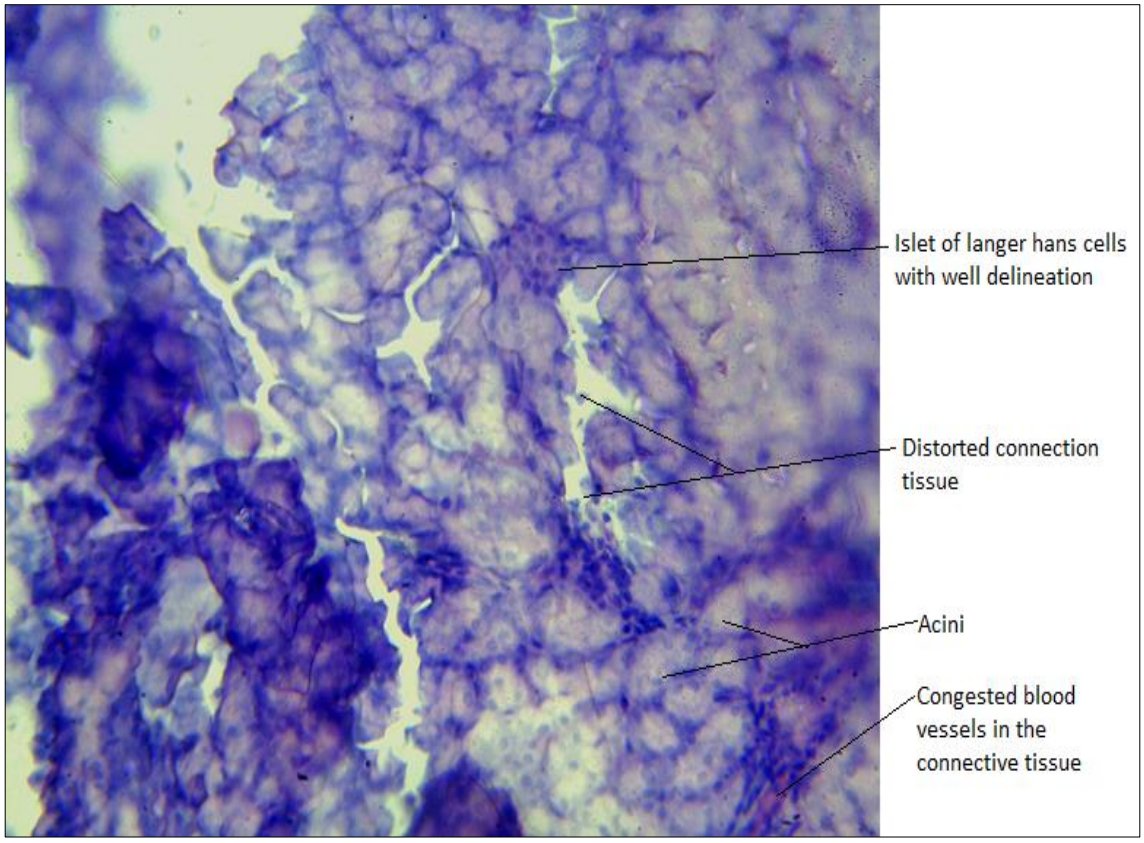

Plate 5: Photomicrograph of pancreatic tissue of hyperglycaemic Wistar albino rat treated with 1000 mg/kg BW of extract, H & E, X400. Plate shows well delineated islet of Langerhans, congested vessels in the connective tissue, distorted interstitial tissue space and normal acini with no intraluminal secretion

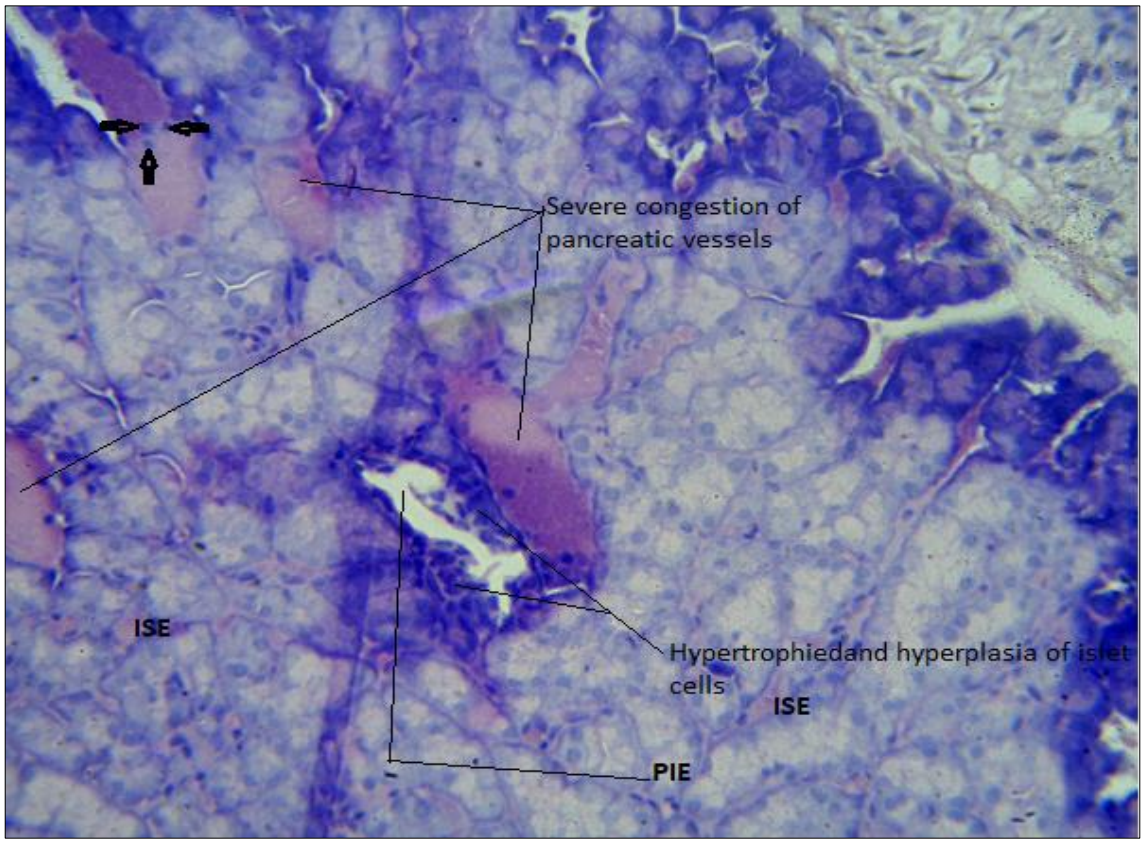

Plate 6: Photomicrograph of pancreatic tissue of hyperglycaemic Wistar albino rat treated with 1500 mg/kg BW of extract, H & E, X400. Plate shows severe congestion of pancreatic vessels with lymphatic cells (arrowed) deposit, interstitial space edema (ISE) and pancreatic islet edema (PIE)

The aim of this research was to determine whether or not administering an ethanol extract of *Andrographis paniculata* leaves to Wistar albino rats induced with alloxan would have any influence on certain biochemical measures. A reduction in endogenous insulin release and therefore impaired glucose uptake by tissues are responsible for the diabetic state induced by alloxan monohydrate (Pari and Maheswari, 1999; Mooradian, 2009) [45, 41]. Table 1 shows general weight loss in all groups after inducement. While normal and positive control groups (groups 1 and 2) continued to lose weight post-treatment, rats given extracts at 1500 mg/kg BW concentration exhibited a significant ($p \leq 0.05$) gain. Extreme weight loss, likely related to muscular atrophy, is diagnostic of diabetes type-1 and loss of insulin production leads to profound catabolism with increased gluconeogenesis, glycogenolysis, lipolysis, and muscle proteolysis causing hyperglycaemia and osmotic diuresis (Farida and Shoukry, 1988; Castellanos *et al.*, 2020) [24, 15]. Elevated counter-regulatory hormone levels promote increased ketogenesis and release "ketone bodies" into the bloodstream, which break down to release hydrogen ions and produce a severe acidosis. The three main symptoms of this illness are ketoacidosis, hyperglycaemia, and dehydration (Castellanos *et al.*, 2020) [15]. Weight loss in this research was consistent with that reported by Pari and Maheswari (1999) [45] and Castellanos *et al.*, (2020) [15], who hypothesized that the incapacity of muscle tissue to metabolize blood glucose caused the breakdown of protein and fat reserves to provide fuel. In contrast, the extract caused both statistically and non-significant dose-dependent increases in relative rat body weight. These findings support those of Alimohammadi *et al.* (2013) [3] and Miaffo *et al.* (2019) [40]. The increased relative body weight of the rats, as reported by Whitton and Hems (1975) [58], is an indication of its influence on the prevention of muscular atrophy.

All extract-treated groups (groups 4 to 6) had significantly lowered fasting blood glucose levels than the negative and positive control groups (Table 2; all $p \leq 0.05$). The extract's flavonoids, phenols, and glycosides may mimic insulin or boost its synthesis by the islets of Langerhans' β-cells, which may explain these results (Tanko *et al.*, 2008; Khurshid *et al.*, 2022) [53, 31]. According to Burcelin *et al.* (1995) [14], an extract may regenerate cells in the islets of Langerhans, transport blood glucose in peripheral tissue, stimulate glucose uptake, inhibit endogenous glucose production, or activate glycogenesis in the liver and muscles.

Lipid profile results in Table 3 revealed a significant decrease ($p \leq 0.05$) in TC, TG, LDL-C and VLDL-C levels of all the extract treated groups (groups 4 to 6) when compared to the normal control and a non-significant increase ($p \geq 0.05$) in HDL-C levels in extract treated groups when compared to the negative control group. These findings suggest that the extract had a positive effect on the diabetic animals' lipid profiles. Increased levels of fasting and postprandial triglycerides, decreased levels of HDL-cholesterol, increased levels of LDL-cholesterol, and a preponderance of tiny, dense LDL particles are all hallmarks of diabetic dyslipidaemia, as described by Wu and Parhofer (2014) [59]. These lipid alterations, Wu and Parhofer (2014) [59] noted, are the primary mechanism through which diabetes contributes to diabetic individuals' elevated cardiovascular risk. Insulin resistance and β-cell dysfunction may be induced by free fatty acids, which are in turn caused by increased triglycerides (Lee *et al.*, 1994; Briaud *et al.*, 2001) [34, 13]. Although the precise processes are still poorly known, as noted by Rachek (2014) [46], it seems that elevated levels of free fatty acids impede normal β-cell function by disrupting or modifying the cascade linking insulin receptors with glucose transporters. Higher HDL-C concentrations were linked to lower hyperglycaemia in a trial assessing the cholesterylester transfer protein inhibitor torcetrapib conducted by Barter *et al.* (2011) [10]. The animals' diet may account for the little but noticeable rise in HDL-C levels shown in this research. HDL is made when triglyceride-rich lipoproteins are broken down in the liver which is also generated in the liver and intestines (Josefa *et al.*, 2019) [29]. The cholesterol level of HDL (HDL-C) is used as a measurement of HDL in Clinical Chemistry. A lower likelihood of cardiovascular disease has been associated to higher plasma levels of high-density lipoprotein (HDL-C) (Angelantonio *et al.*, 1993; Rader and Hovingh, 2014) [7, 47]. Saponins, flavonoids, and phenolic compounds are examples of secondary metabolites that have been shown to have lipid-lowering effect (Limaye *et al.*, 2003; Tungmunnithum *et al.*, 2018) [35, 55]. The presence of these types of phytochemical substances in the extract may account for the reported positive lipidemic action.

Table 4 revealed that there was a significant increase ($p \leq 0.05$) in plasma insulin levels in all the extract treated groups (groups 4 to 6) when compared to the normal control group and significant increase ($p \leq 0.05$) in the treated (500 mg/kg BW of extract) group when compared to the negative control group. Insulin, a peptide hormone synthesized by beta cells in the pancreatic islets, is encoded by the INS gene. Beta cells, which are sensitive to glucose levels, secrete insulin into the blood when blood sugar levels are high and prevent insulin from being secreted when blood sugar levels are low (Koeslag *et al.*, 2003) [33]. *Andrographis paniculata* extract significantly reduced blood glucose and increased plasma insulin levels in diabetic rats, according to the study. The extract may work by a number of different mechanisms, including stimulating glucose uptake by peripheral tissues and blocking the body's ability to make glucose by inhibiting gluconeogenesis in the liver and muscles (Burcelin *et al.*, 1995) [14]. Glucose is stored as glycogen in the liver and muscles through a process called glycogenesis. Insulin increases glycogen synthesis by inhibiting glycogen phosphorylase, as shown by Bhandari *et al.* (2013) [11]. This enzyme is essential for the conversion of glycogen into glucose. Patients with diabetes have significantly diminished glycogen storage in the liver due to insulin resistance and reduced glycogen storage in the liver may lead to postprandial hyperglycaemia in patients with type-2 diabetes mellitus (Bollen *et al.*, 1998; Jiang *et al.*, 2020) [12, 28]. The extract contains chemical components (flavonoids, phenols, and glycosides), phytochemicals that might promote its production by the β-cells of the islets of Langerhans, which may account for the observed effects (Khurshid *et al.*, 2022) [31].

Figure 1 showed the percentage inhibitory potency of ethanol extract of *Andrographis paniculata* leaves on α-glucosidase activity. The results showed appreciable alpha-glucosidase inhibitory effect which was highest at the lowest dose of 500 mg/kg BW. Percentage inhibition somewhat dipped with increased concentration though non-significantly. None of the extract doses was able to attain percentage inhibition above 50% (IC_{50}). Carbohydrate

metabolism relies heavily on the enzyme alpha glucosidase. It is responsible for the hydrolysis of alpha-1,4-glucosidic linkages in oligosaccharides and disaccharides, which ultimately results in the release of glucose. The activity of alpha glucosidase is critical for maintaining normal blood glucose levels in humans. This study's findings corroborate that of Subramanian *et al*. (2008) [52], who studied the *in-vitro* effects of *Andrographis paniculata* ethanol leaf extract on the pancreatic alpha-glucosidase activity of streptozotocin-induced diabetic rats. The extract was shown to inhibit alpha-glucosidase in a concentration-dependent manner (IC50 =17.2+/-0.15 mg/ml). The flavonoid concentration of the extracts was found to be 0.022 g/mL quercetin with an Rf value of 0.61 by Fardiyah *et al*. (2020) [23], who studied the potential and characterisation of *Andrographis paniculata* L. Ness plant extracts as a photoprotective agent. At a retention time of 2.77 on the LCMS/MS chromatogram, quercetin-3-glycoside standard shows up with a comparable appearance to the sample. According to Fardiyah *et al*. (2020) [23], the quercetin-3-glycoside (isoquercitrin) content in *A. paniculata* plant extracts was 9.17 µg/mL. Twenty-one different flavonoids were investigated for their ability to inhibit alpha-glucosidase (EC 3.2.1.20) and alpha-amylase (EC 3.2.1.1) by Kim *et al*. (2000) [32]. The authors found that luteolin, amentoflavone, luteolin 7-O-glucoside, and daidzein performed as the best inhibitors of α-glucosidase activity. The possibility of plant extracts to regulate hyperglycaemia was highlighted by Nair *et al*. (2013) [43] due to their action as alpha-glucosidase inhibitors. The blocking of alpha-glucosidase may be a possible mechanism by which the *A. paniculata* extract exerts anti-diabetic action, and the significant decrease in fasting blood glucose levels as seen in all extract-treated groups indicates that the extract might be an encouraging choice for treating diabetes mellitus.

Degeneration of pancreatic islet cells (pygnosis) was observed in diabetic negative control rats in Plate 3. Insulin insufficiency probably originated from this trait. Hyperglycaemia is the result of insulin insufficiency (or diabetes mellitus), which leads to the abnormal increase and inefficient use of blood glucose (Ezejiofor *et al*., 2013) [22]. Histopathological analysis of diabetes treatment groups revealed a potentially regenerative increase in islet volume density and beta cell percentage in diabetic rats given the extracts. Excessive stimulation and increasing demand may trigger the regeneration processes of hyperplasia (cell number increase) and hypertrophy (cell size increase). Due to β-cell destruction by alloxan, hyperglycaemia is induced. If left unchecked (as in plate 3), there is continual and gradual necrosis of the islet cells. Beta cell destruction may include the adaptive immune system, since people with type-1 diabetes likely to have more CD8+, T-cells, and B-cells that selectively target islet antigens than those without type-1 diabetes (DiMeglio *et al*., 2018) [19]. Both necroptosis and apoptosis, caused or worsened by CD8+ T-cells and macrophages, are likely involved in the beta cells' true death process (Atkinson *et al*., 2020) [8]. Directly by the release of toxic granzymes and perforin from activated T-cells, or indirectly through the reduction of blood flow or the production of reactive oxygen species (Atkinson *et al*., 2020) [8], necroptosis may be initiated. Atkinson *et al*. (2020) [8] found that when certain beta cells die, they may produce biological components that enhance the immunological response, leading to even more inflammation and cell death. Beta cell apoptosis, which is connected to activation of the janus kinase and TYK2 pathways (Atkinson *et al*., 2020) [8], also exists in the pancreas of people with type-1 diabetes. Some plant extracts have been shown to reduce blood glucose levels, show signs of β-cell regeneration, and enhance insulin production from surviving β-cells in the islets of Langerhans (Shanmugasundaram *et al*., 1990; Abdel Moneim *et al*., 1997) [50, 1]. Higher concentrations of the *Andrographis paniculata* leaf extract had a greater restorative effect on diabetic rats' islet cells than lower doses, suggesting that it may have chemical components that regenerate β-cells and stimulate them to produce more insulin (pancreatotrophic action), or contain insulin-like substances (Nagajothi *et al*., 2018) [42].

Conclusion

From the findings of this study, it can be concluded that alloxan-induced hyperglycaemic Wistar albino rats showed a substantial decrease in fasting blood glucose levels when treated with an ethanol extract of *Andrographis paniculata*. This may have been achieved by the inhibition of plasma α-glucosidase enzyme activity and the induction of insulin secretion from pancreatic beta cells. Hence, *Andrographis paniculata* extracts can serve as a promising novel herbal antidiabetic agent. Further studies on hyperglycaemia, both *in-vivo* and *in-vitro* should be carried out using higher concentrations or doses of the leaf extracts as well as using different solvents for the extraction process. Other parts of the plant should also be investigated for possible therapeutic effect on animal model.

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