

The hypoglycemic effect of purple sweet potato leaf fractions in diabetic rats

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ABSTRACT

There is considerable interest in herbal medicine in developing antidiabetic alternative therapy based on plant-derived compounds that would reduce oral hypoglycemic agent-related adverse effects. *Ipomoea batatas* L. leaf has been reported as rich in phenolic compounds that can reduce the hyperglycemic condition. Research on the hypoglycemic effect of *I. batatas* L. leaf fraction in alloxan-induced diabetic rats has been conducted. Fractionation methods included gradual maceration with n-hexane, ethyl acetate, and ethanol. The diabetic condition was used alloxan intraperitoneally in rats until blood glucose levels reached > 200mg/dL. Rats were divided into 12 groups that are normal control, diabetic control, insulin treatment, n-hexane fraction (HF), ethyl acetate fraction (EAF), and ethanol fraction (EF) with doses of 50, 100, and 200 mg/kg BW, respectively. Blood glucose level and pancreatic homogenate MDA level were evaluated spectrophotometrically. Histopathology of pancreatic tissues was performed using hematoxylin-eosin stain. According to the findings of this investigation, the decrease in blood glucose level and pancreatic homogenate MDA level of each fraction increased with increasing dosage. The best result was shown: the ethanolic fraction with a dose of 200 mg/kg BW has not significantly different ($p>0.05$) from the insulin treatment group in decreasing blood glucose levels and pancreatic homogenate MDA levels. The histopathological observation results showed the dose of 200mg/kg BW of an ethanolic fraction of *I. batatas* L. leaf had the best improvement pancreatic tissues.

Keywords: Alloxan, Fraction, *Ipomoea batatas* L, MDA

Introduction

According to the 2021 report of the IDF (International Diabetes Federation), 573 million individuals worldwide have diabetes, with 700 million cases anticipated by 2045 [1]. Globally, this illness is more widespread in low- and middle-income countries, where more than 50% of cases go misdiagnosed. As sweet potatoes have shown promise as economical anti-diabetes drugs, they can be utilized as a possible diabetes therapy. The phytochemicals phenolic acids, anthocyanins, flavonoids,

saponins, glycosides, alkaloids, and terpenes [2, 3] contribute to this. These phytochemicals exhibit an anti-diabetic impact through a number of methods, including insulin mimicking action, beta-cell stimulation to produce insulin, adjustment of metabolic parameters, enzyme inhibitory activity, and/or modification of glucose utilization [4].

According to scientific research, purple sweet potato leaves can reduce blood glucose levels. In vivo studies have shown the effectiveness of purple sweet potato leaf extract in reducing insulin resistance and controlling blood sugar levels. It was demonstrated that water extracts of purple sweet potato leaf decreased fasting blood glucose levels in STZ-induced diabetic rats [5]. In a similar manner, Nagamine *et al.* [6] demonstrated that consuming sweet potato leaf extract can decrease hyperglycemia in KK-Ay mice, the mechanism is by boosting the synthesis of glucagon-like peptide-1 (GLP-1). An extract of purple sweet potatoes leaves increased glucose absorption by elevating Glut4 expression in 3T3-L1 adipocyte cells and altering the PI3K/AKT signaling pathway [7]. In addition, Shih *et al.* [8]

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discovered that the leaves of white-fleshed sweet potato significantly decreased blood glucose and lipid levels and also increased insulin signaling pathway expression in mice with STZ-induced diabetes.

The anti-diabetic activity of sweet potato leaf is typically attributed to phytochemicals, such as phenolic acids, flavonoids, and anthocyanin [2, 6, 9]. The *in vitro* studies reported that the sweet potato leaf extract can inhibit the α -amylase and α -glucosidase enzymes, which convert starch and oligosaccharides into monosaccharides [2, 9]. To the best of our knowledge, this is the first report on the evaluation of purple sweet potato (*Ipomoea batatas* L.) leaf fraction (polar, semi-polar, and non-polar) for hypoglycemic activity in alloxan-induced diabetic rats.

Materials and Methods

Reagents and chemicals

Alloxan monohydrate, GOD-PAP, Ethanol, Ethyl acetate, n-Hexane, TEP (1,1,3,3-tetra ethoxy propane), insulin, thiobarbituric acid (TBA), trichloro acetic acid (TCA)

Plant collection

Leaves of *Ipomoea batatas* L. were collected from Lubuk Linggau, South Sumatera, Indonesia. Identification of plants was done by the Plant Conservation Centre, Purwodadi Botanical Garden, Indonesian Institute of Sciences, Purwodadi, Central Java, Indonesia. The authentic sample with voucher number 830/IPH.06/HM/VIII/2019.

Extraction and fractionation

The leaves of purple sweet potato (*Ipomoea batatas* L.) were dried at 40°C in an oven and then ground into a powder. The process of soaking 1.5 kg of leaf powder in 15 L of n-hexane was repeated until the solvent became clear. The resulting extract was filtered and then vacuum-dried at 60°C in a rotary evaporator to produce viscous material (HF). Using the same technique, 15L of ethyl acetate (EAF) and 15L of ethanol (EF) were macerated with the powdered pulp, respectively.

Phytochemical screening

Identification of flavonoids

0.5 g of samples were dissolved in 5 mL of hot ethanol for five minutes. The filtrate was then treated with a few drops of concentrated HCl and 0.2 mg of magnesium powder (Mg metal). The presence of flavonoid group compounds was indicated by the color red [10].

Identification of saponin

One to two grams of samples were boiled for two minutes and vigorously shaken in distilled water. In a test tube, the formation of stable foam indicated the presence of saponins [10].

Identification of alkaloids

Two grams of samples were collected, then ammonia dissolved in chloroform was mixed with a small amount of chloroform and sand. The mixture was stirred prior to filtration. The filtrate was then blended with 2N H₂SO₄ and vigorously shaken. Two distinct layers developed. Mayer, Wagner, and Dragendorff reactors were used to analyze the water-phased top layer. The presence of alkaloids was indicated by red sediment deposits with Wagner reagents, white sediment deposits with Mayer reagents, and a red or orange hue with Dragendorff reagents [10].

Identification of steroids and triterpenoids

The lowest layer removed from the identification of alkaloids was placed on a drop plate to dry. Two drops of anhydrous acetic acid and one drop of concentrated sulfuric acid were added (Lieberman-Buchard reagent). The presence of a green or red hue indicates the presence of steroids or triterpenoids [10].

Identification of tannins

Two grams of samples were mixed with one hundred milliliters of water, heated for fifteen minutes, cooled, and filtered to produce filtrate. A 1% FeCl₃ solution was added to the filtrate. The presence of tannin compounds was indicated by pigmentation that was dark blue or blackish green [10].

In vivo experimental

Animal preparation

Male Wistar rats measuring 150-200 g and 8-10 weeks old were employed, and they were acclimated for seven days in the laboratory. All animals were cared for in accordance with authorized animal care protocols, as certified by the Ahmad Dahlan University research ethics committee (certificate number 022012047). They were fed a conventional pellet rat diet and had access to water at will. They were housed in a polypropylene cage with a 12h light-dark cycle at a temperature of 25±2°C.

Induction of diabetes

Wistar male rats were utilized to test the efficacy of HF, EAF, and EF in alloxan-induced diabetic rats. Alloxan monohydrate (130 mg/kg BW) dissolved in 0.9% saline was administered intraperitoneally to rats that had fasted for 15 hours (except for the normal control group). After 72 hours of alloxan injection in rats that had fasted for 12 hours, each rat's glucose level was measured, and only rats with glucose levels above 200 mg/dL were included in the diabetic group experiment. Twelve groups of diabetic animals were dispersed (5 rats in each). In this experiment, the rats were fed their regular diet. The HF, EAF, and EF were dissolved in 1% Sodium CMC and animals were administered the following treatment protocol:

Group 1: Normal control group received 1 ml/kg BW of 1% Sodium CMC per oral

- Group 2: The diabetic control group received 1 ml/kg BW of 1% sodium CMC per oral
- Group 3: Diabetic positive control group received insulin 1 IU/kg BW in 0.9% saline subcutaneously
- Group 4: Diabetic rats received HF (50mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 5: Diabetic rats received HF (100mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 6: Diabetic rats received HF (200mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 7: Diabetic rats received EAF (50mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 8: Diabetic rats received EAF (100mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 9: Diabetic rats received EAF (200mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 10: Diabetic rats received EF (50mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 11: Diabetic rats received EF (100mg/kg BW) dissolved in 1% Sodium CMC per oral
- Group 12: Diabetic rats received EF (200mg/kg BW) dissolved in 1% Sodium CMC per oral

Body mass and fasting glucose levels were measured every 5 days. Animals were euthanized by cervical dislocation after treatment completion for histopathological study.

Assessment of antidiabetic activity

Rats were fed their regular diet for 15 days while receiving their different treatments. Every five days, the level of fasting blood glucose in rats was determined spectrophotometrically from the plexus retro-orbitalis using the GOD-PAP reagent [11]. The animals were euthanized under light ether anesthesia on the fifteenth day. The pancreas was removed and washed in a cold saline solution. A portion of the pancreas was evaluated for MDA concentration and histology.

Homogenate preparation of pancreas

Fresh pancreatic organs are cut into small pieces and then crushed using a mortar under cold conditions in 2.5 mL of PBS solution containing 11.5 g/L KCl by placing them on a block of ice. The homogenate was transferred to an Eppendorf tube and centrifuged at 4,000 rpm for ten minutes. The supernatant was then removed and transferred to an Eppendorf tube. Add 0.5 mL of sample or standard to 2 mL of cold 0.25 N HCl mixture containing 0.38 % thiobarbituric acid (TBA), 0.5% butylated hydroxytoluene (BHT) and 15% trichloroacetic acid (TCA). This combination was heated for one hour at 80°C. The solution and reference combination was centrifuged at 3,500 rpm for 10 minutes after cooling. The obtained supernatant was utilized for the estimation of MDA concentration.

Lipid peroxidation assay

The method of Mendes *et al.* (2009) was utilized for lipid peroxidation evaluation. TEP (1,1,3,3-tetra ethoxy propane)

was utilized as the MDA standard without hydrolysis prior to the TBA reaction. A standard curve was generated using TEP diluted in 7.5% TCA solution at concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 M. 5 ml of sample supernatant, standard, or blank was placed in a screw-capped tube, 5 ml of 20 mM TBA solution was added, and the mixture was rapidly agitated for 60 minutes at boiling temperature in a water bath. The MDA-TBA combination was measured spectrophotometrically at 532 nm after cooling. Results were reported as micromoles of MDA per liter of homogenate [12, 13].

Histopathological examination

To assess any changes in pancreatic beta-cells following treatment, the pancreas of euthanized rats was harvested. The group's pancreatic tissues were collected in formalin containing 10% neutral buffered phosphate, then processed and embedded in paraffin wax. Using a microtome, 5µm thick sections were cut and stained with hematoxylin-eosin. The slices were inspected microscopically to assess histological alterations.

Statistical analysis

The values were expressed as the mean \pm standard deviation. SPSS 26 was used to conduct a one-way analysis of variance on the differences between different treatments for in vivo research. Duncan's multiple comparison tests determined, at P 0.05, that there was a statistically significant difference between the groups.

Results and Discussion

Fractionation and phytochemical screening

Fractionation of *Ipomoea batatas* L. leaf by gradual maceration using n-hexane, ethyl-acetate, and ethanol, respectively. The yield of each fraction can be seen in (Table 1).

Table 1. The yield of *Ipomoea batatas* L. leaf fraction

Fraction	Total (g)	Yield (%)
n-Hexane	36.800	2.450
Ethyl-acetate	18.500	1.233
Ethanol	94.900	6.330

The percentage of yield obtained showed that the ethanolic fraction had the highest value (6.330%) compared to the other fractions. Ethanol is a polar solvent, which can attract polar compounds. These results indicate that the polar compound in *Ipomoea batatas* L. leaf was dominant.

Phytochemical screening is a qualitative assessment of a plant or extract's chemical composition to guarantee the presence of the desired secondary metabolite. The findings indicate the presence of several components in each fraction. The findings of phytochemical tests are shown in (Table 2).

Table 2. Phytochemical compound of *Ipomoea batatas* L. leaf fraction

Group Compound	Fresh leaf	n-Hexane fraction	Ethyl-acetate fraction	Ethanol fraction
Alkaloid	+	+	-	+
Flavonoid	++	-	++	+
Tannin	++	-	+	++
Saponin	-	-	-	+
Steroid	+	+	-	-
Triterpenoid	+	++	-	-

Ethanol fraction has contain more phytochemical compounds than other fractions of *Ipomoea batatas* L. leaf.

Antidiabetic evaluation

Alloxan-induced diabetes

Baseline blood glucose level was determined before alloxan-monohydrate injection. All rats showed normal blood glucose levels with no significant difference among the groups as seen in (Table 3).

Table 3. Blood glucose level (mg/dL) of rats before and after induction with alloxan monohydrate 130mg/kg BW

Groups	Blood glucose level (mg/dL)	
	Before induction (mean±SD)	After induction (mean±SD)
Normal	110.578±3.242	111.125±2.375
Diabetic	114.394±2.769	276.393±9.053***
Insulin	104.938±3.042	217.315±10.312***
HF50	100.450±4.816	229.533±3.459***
HF100	95.528±4.134	246.003±4.929***

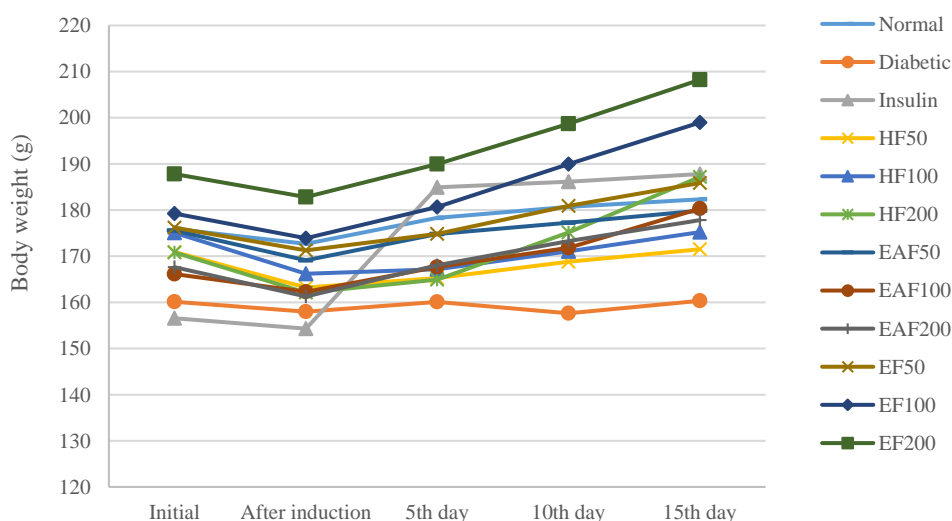
HF200	100.620±5.955	255.265±8.560***
EAF50	111.828±1.219	251.068±1.394***
EAF100	108.500±0.977	242.620±1.877***
EAF200	104.355±2.137	231.380±2.786***
EF50	96.038±9.911	251.540±7.197***
EF100	114.513±2.651	247.650±4.512***
EF200	103.470±6.962	245.280±4.512***

Note : *** = blood glucose level before and after induction is significantly different (P < 0.0001)

The successful diabetic induction with alloxan monohydrate intraperitoneally was marked by a sudden increase in blood glucose serum level at > 200 mg/dL. Three days after alloxan injection, all alloxan-induced groups (except the normal group), have blood glucose levels > 200 mg/dL (Table 3). No apparent signs of toxicity were noted, including convulsions, restlessness, agitation, respiratory distress, or coma. All animals were still alive after 15 days of monitoring.

The effect of *Ipomoea batatas* L. leaf fractions on body weight

Alloxan is the main compound used to induce type 1 diabetes mellitus models in animal studies that contribute to the functional defect of pancreatic beta-cells and then lead to hyperglycemia and weight loss. Type 1 diabetes mellitus illness has resulted in a drop in body weight as a result of insulin shortage, which hinders the use of glucose for energy sources and encourages the consumption of stored fats and muscle protein for energy. After alloxan injection, all groups were significantly reduced in body weight (p < 0.0001), except the normal group (Figure 1).

**Figure 1. Effect of *Ipomoea batatas* L. leaf fractions on rat's body weight**

After treatment for the first 5 days, all groups saw an increase in body weight with time (P < 0.001), except for the diabetic group. After 15 days, all treatment groups gained body weight to normal levels. The best body weight gain, reach by ethanol

fraction dose 200 and 100 mg/kg BW treatment. This data demonstrates that a dose of 200 mg/kg BW of *Ipomoea batatas* L. leaf ethanol fraction had the best action in preventing muscle

wasting due to hyperglycemic status and improved metabolic activity.

The effect of Ipomoea batatas L. leaf fractions on the blood glucose level.

The diabetes-induced group had a blood glucose level of > 200 mg/dL, which was considerably higher than the serum glucose level of the normal group ($p < 0.0001$). The decrease in blood

glucose level that began on the 5th-day treatment was significantly different from the diabetic group ($p < 0.001$). On the 10th-day treatment, the ethanol fraction dose 200 mg/kg BW group had reached normal blood glucose level, meanwhile the positive group (receive insulin 1 IU/kg BW) on the 15th day. The groups that receive n-hexane fraction dose 50 and 100 mg/kg BW and ethyl-acetate fraction dose 50 and 100 mg/kg BW has not reached normal blood glucose levels on day 15. The complete data can be seen in (Table 4).

Table 4. Comparison of blood glucose level (mg/dL) among groups

Groups	Blood glucose level (mg/dL) (mean±SD)			
	Initial day	5 th day	10 th day	15 th day
Normal	111.125±2.375 ^a	110.955±4.879 ^a	109.143±7.309 ^a	107.640±9.862 ^{cd}
Diabetic	276.393±9.053 ^e	273.393±14.068 ^e	273.485±12.655 ^e	278.197±14.092 ^j
Insulin	217.315±10.312 ^b	185.513±13.686 ^b	145.737±11.784 ^d	102.660±6.424 ^{bc}
HF50	229.533±3.459 ^b	220.498±5.523 ^d	202.270±5.996 ^h	176.400±8.107 ⁱ
HF100	246.003±4.929 ^{cd}	214.655±8.498 ^d	185.970±8.016 ^g	149.713±7.596 ^h
HF200	255.265±8.560 ^d	206.045±11.311 ^{cd}	165.233±7.949 ^e	122.027±6.290 ^{ef}
EAF50	251.068±1.394 ^d	223.170±2.745 ^d	181.393±6.297 ^g	138.858±1.482 ^{gh}
EAF100	242.620±1.877 ^{cd}	216.203±3.889 ^d	175.683±4.055 ^{ef}	130.890±2.539 ^g
EAF200	231.380±2.786 ^{bc}	206.003±6.772 ^{cd}	168.893±11.877 ^{ef}	111.405±2.729 ^{bc}
EF50	251.540±7.197 ^d	213.785±12.278 ^d	132.933±8.476 ^{cd}	98.625±2.147 ^{bc}
EF100	247.650±4.512 ^{cd}	189.490±9.598 ^{bc}	128.358±7.229 ^{bc}	93.200±3.070 ^{ab}
EF200	245.280±4.512 ^{cd}	177.003±19.900 ^b	115.088±8.193 ^{ab}	83.273±2.616 ^a

Note: numbers in the same column followed by the different superscript letter (^{a,b,c}) are significantly different ($p < 0.05$)

The percentage reductions in blood glucose levels are shown to determine the capability of each fraction of *Ipomoea batatas L.* leaf as a new antidiabetic candidate. As seen in (Table 5), all treatment groups exhibited a > 20% reduction in blood glucose levels for 15 days of treatment. The percent reduction of blood glucose level is presented in the following descending order: EF200>EF100>Insulin>EF50>EAF200>HF200>EAF100>HF100>EAF50>HF50. The groups that receive an ethanol fraction dose of 100 mg/kg BW have a similar percent reduction of blood glucose levels to the positive group (receive insulin 1 IU/kg BW) ($p > 0.05$). While the group that receives ethanol fraction dose 200 mg/kg BW treatment has a greater percent reduction in blood glucose level than the positive control group.

Table 5. Percent reduction in blood glucose level for 15 days of treatment

Groups	Percent Reduction (%) (mean±SD)
Diabetic	2.887±0.492 ^g
Insulin	40.433±4.364 ^{ab}
HF50	24.124±2.173 ^f
HF100	27.428±2.747 ^{ef}
HF200	32.097±3.347 ^{cde}
EAF50	27.302±1.191 ^{ef}
EAF100	29.834±1.064 ^{de}
EAF200	33.757±2.708 ^{cd}

EF50	36.727±3.039 ^{bc}
EF100	40.792±2.479 ^{ab}
EF200	44.661±4.021 ^a

Note: numbers in the same column followed by the different superscript letter (^{a,b,c}) are significantly different ($p < 0.05$).

Defensive effect of Ipomoea batatas L. leaf fractions on pancreatic lipid peroxidation

The amount of MDA increased in the pancreas of alloxan-induced diabetic rats compared to the normal control group. Insulin, HF200, EF50, EF100, and EF200 administration restored the MDA level of normal control rats in a dose-dependent manner. The treatment of EF200 was more successful than insulin therapy in restoring the level of MDA (Table 6).

Table 6. Comparison of MDA pancreatic homogenate level among groups

Groups	MDA level (μmol/L) (mean±SD)
Normal	1.930±0.072 ^{ab}
Diabetic	6.010±0.461 ^c
Insulin	1.925±0.142 ^{ab}
HF50	2.663±0.122 ^{bc}
HF100	2.515±0.116 ^{abc}
HF200	2.125±0.074 ^{ab}

EAF50	4.145±1.038 ^d
EAF100	2.890±0.714 ^c
EAF200	2.200±0.267 ^{abc}
EF50	2.045±0.045 ^{ab}
EF100	2.015±0.059 ^{ab}
EF200	1.845±0.029 ^a

Note: numbers in the same column followed by the different superscript letter (^{a,b,c}) are significantly different ($p < 0.05$)

Defensive effect of *Ipomoea batatas L.* leaf on histopathology of the pancreas

The histoarchitecture of the pancreas was depicted in **Figure 2**. The normal control group displayed compact islets of Langerhans

surrounded by intact normal acinar cells with conspicuous nuclei. Normal controls possessed a pancreatic duct devoid of inflammation or degeneration. Langerhans exhibited loss of islets, infiltration of islet cells by inflammatory cells, and significant cytoplasmic vacuolation of islet cells in diabetic control rats. Acute deformation and rupture of acinar cells and acinar cell steatosis were also seen. The administration of insulin (1 IU/kg BW) to diabetic rats had no discernible impact on reducing pancreatic damage. Administration of EF to diabetic rats restored dose-dependently the pathological alterations observed in normal control rats. The protective effect of EF 200 mg/kg BW on the histology of the pancreas was more prominent in diabetic rats.

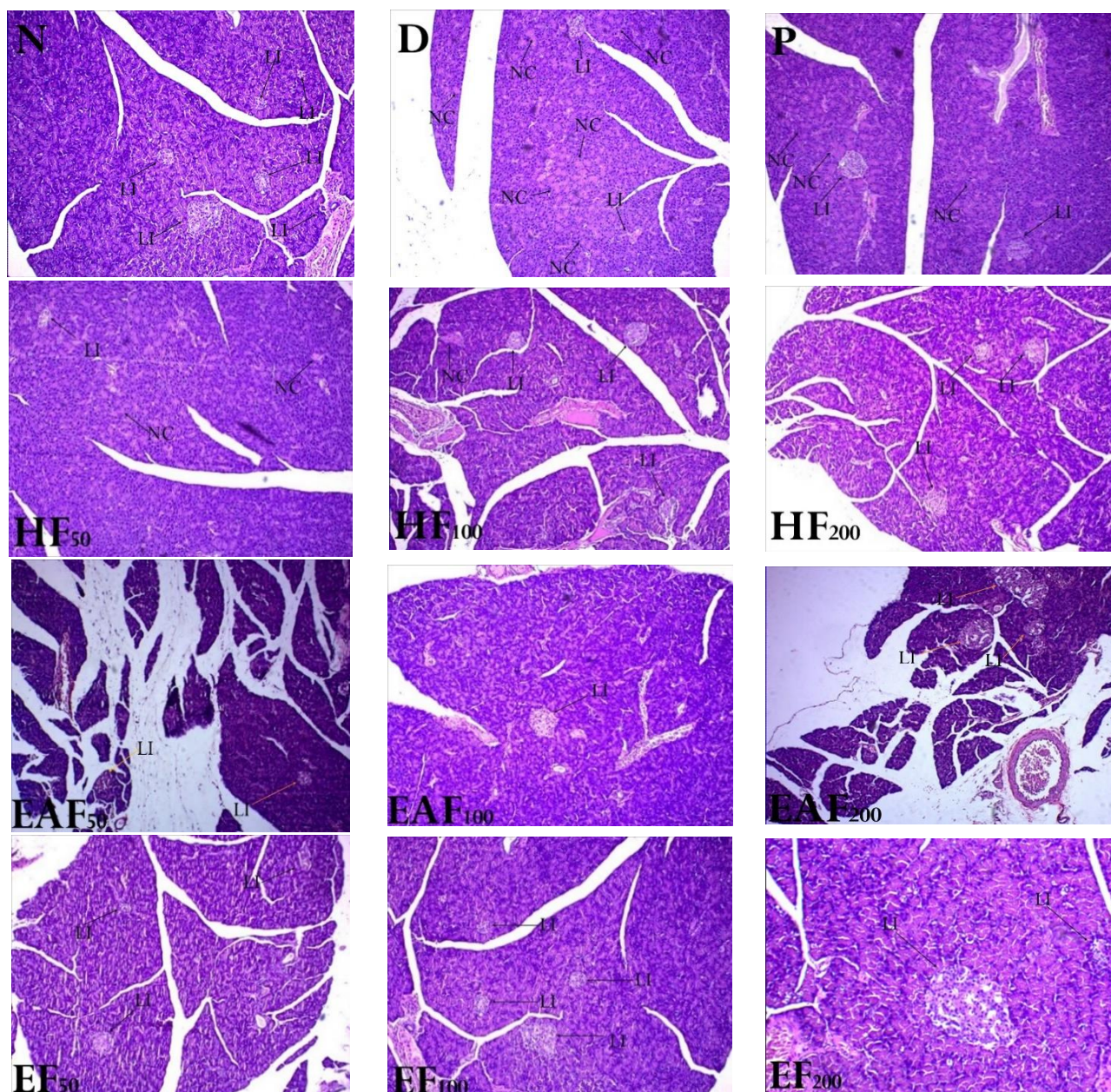


Figure 2. Histopathological features of pancreatic tissue (N: Normal group, D: Diabetic group, P: positive group receive insulin 1 IU/kg BW, HF50: receive n-hexane fraction 50 mg/kg/ BW, HF100: receive n-hexane fraction 100 mg/kg BW, HF200: receive n-hexane fraction 200 mg/kg BW, EAF50: receive ethyl-acetate fraction 50 mg/kg BW, EAF100: receive ethyl-acetate fraction 100 mg/kg BW, EAF200: receive ethyl-acetate fraction 200 mg/kg BW, EF50: receive ethanol fraction 50 mg/kg BW, EF100: receive ethanol fraction 100 mg/kg BW, EF200: receive ethanol fraction 200 mg/kg BW) (LI = Langerhans Islet; NC = Necrotic Cell).

In this experiment, diabetes was induced by intraperitoneal injection of alloxan-monohydrate, which induces a functional defect in pancreatic beta cells, resulting in hyperglycemia, weight loss, and decreased endogenous insulin production. Moreover, it affects glucose levels by inhibiting insulin production by beta cells in the islet of Langerhans [14]. Rats injected with alloxan develop a type of insulin-dependent diabetes mellitus known as alloxan-induced diabetes [15]. Alloxan's diabetogenicity is highlighted by its preferential absorption by beta cells of the pancreas, which is enhanced by Glut2. Due to the similarities in their chemical structures, glucose and alloxan compete for the same transport protein [16]. Evaluation of the glucose level after induction confirmed that all animal groups, with the exception of the normal control group, were in a diabetic state, with glucose levels exceeding 200 mg/dL (**Table 3**).

Due to insulin deficiency, which impedes the use of glucose as an energy source and stimulates the consumption of stored lipids and muscle protein for energy, diabetes causes weight loss. Our observations showed that administration of *Ipomoea batatas* L. leaf fractions significantly and dose-dependently raised body weight. The rats in the EF 200 mg/kg BW group gained the highest weight relative to the other treatment groups (**Figure 1**). The rise in body weight suggests that the ethanol component of *Ipomoea batatas* L. leaf might prevent muscle loss caused by a hyperglycemic state and enhance metabolic activity.

Moreover, our data suggested that the n-hexane, ethyl acetate, and ethanol fractions of *Ipomoea batatas* L. leaf could lower blood glucose levels. Rats who received 200 mg/kg BW of ethanol fraction had the greatest reduction in blood sugar levels, and this was better than the group treated with insulin at 1 IU/kg BW. Previous studies have reported the hypoglycemic properties of *Ipomoea batatas* L. leaf. [17-22]. The phytochemical content as seen in (**Table 2**) shows that the ethanol fraction contains alkaloids, flavonoids, and tannins. Another polar compound found in *I. batatas* L. leaf is anthocyanin glycoside. The predominant anthocyanin aglycones in the leaves of *I. batatas* are cyanidin and peonidin. *I. batatas* mainly contain Peonidin-caffeoyl-feruloyl-sophoroside-5-glucoside, a peonidin derivative. In rats' small intestines, this molecule modulates glycemia by inhibiting maltase-glucoamylase (MGAM) [23]. Limiting the amount of sugar in the blood by inhibiting sugar absorption in the intestines ensures glucose uptake in cells and prevents autooxidation. Cyanidin-3-Rutinoside reduces carbohydrate absorption and, as a result, blood glucose levels by inhibiting alpha-glucosidase in the intestine [24].

In addition, alloxan is a very unstable molecule, which permits it to undergo redox cycling rapidly. Alloxan generates reactive oxygen species (ROS) such as hydroxyl radical ($\cdot\text{OH}$) and superoxide radical anion (O_2^-) in the presence of intracellular thiols, particularly glutathione (GSH), via the autooxidation of its reduction product, dialuric acid [25]. It is assumed that the hydroxyl radical is the most hazardous radicals in the cell and is primarily accountable for β -cell toxicity and the diabetogenicity of alloxan. Damage caused by reactive oxygen species to pancreatic β -cells has been linked to DNA fragmentation, which promotes poly ADP-ribose polymerase 1, a critical enzyme in the

DNA repair process. This phase of alloxan diabetogenicity is caused by the inhibition of insulin release from pancreatic beta cells owing to reactive oxygen species assault [26]. The current data indicates that alloxan administration induced substantial oxidative stress, as evidenced by a considerably greater concentration of MDA pancreatic homogenate compared to the normal control group ($p < 0.0001$). MDA is the most extensively studied and most prevalent byproduct of polyunsaturated fatty acid peroxidation. Since the 1960s, numerous methods have been developed to assess this molecule in order to determine the amount of in vivo and in vitro oxidative stress [27]. Similar investigations have demonstrated that diabetic rats had elevated MDA levels [28, 29]. The *Ipomoea batatas* L. leaf fraction can lower MDA levels in rats to near normal levels, except in the EAF50 group. The treatment with ethanol fraction dosed at 200 mg/kg BW had the lowest MDA level, better than the positive control group (**Table 6**). The antioxidant properties of anthocyanins are attributed to the phenol hydroxyl present in the B-ring aglycone of cyanidin and peonidin, according to reports [30]. Moreover, the antioxidant activity increased when phenolic was added to the acylated residue [30-33]. Anthocyanins with aglycon cyanidin and an acylated caffeoyl residue possess enhanced antioxidant activity due to the catechol structure, specifically the diphenol structure, which donates electrons to LPO radicals more efficiently [30].

Histopathological analysis of diabetic rats revealed pancreatic islets of Langerhans damage. However, the restoration of the number of Langerhans islets in diabetic rats with the ethanol fraction of purple sweet potato leaf may show the regeneration potential of polar chemicals in *I. batatas* L. leaf. In response to physiological stress, the islet of Langerhans is a dynamic structure that can alter in size, number of cells, and molecular activity [34]. Mechanism of β -cell regeneration through trans-differentiation, progenitor differentiation, or proliferation. The proliferation of beta cells is the most direct approach to regenerating beta cell mass. Beta cell regeneration may also target trans-differentiation from other endocrine cells, duct cells, acinar cells, or centro-acinar cells [35]. Previous research indicated that *I. batatas* L. leaf extract protects beta cells in diabetic rats [36]. The mechanism of each chemical in the ethanol fraction of *I. batatas* L. leaf requires more research.

Conclusion

The ethanolic fraction of *Ipomoea batatas* L. dose at 200 mg/Kg BW has a greater effect on the reduction of blood glucose levels and better pancreatic histopathological features than other fractions.

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