

Pancreatic Effects of *Eurycoma longifolia* and *Punica granatum* Combination Extracts in Streptozotocin-Induced Diabetes in Rats

Efectos Pancreáticos de los Extractos Combinados de *Eurycoma longifolia* y *Punica granatum* en la Diabetes Inducida por Streptozotocina en Ratones

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ABDULMUNEM K. O. & AUNG, S. Pancreatic effects of *Eurycoma longifolia* and *Punica granatum* combination extracts in streptozotocin-induced diabetes in rats. *Int. J. Morphol.*, 44(4):1090-1097, 2026.

SUMMARY: Diabetes and obesity are two debilitating public health disorders that frequently occur together and are both on the rise. Regardless, diabetes has become a worldwide epidemic. All forms of diabetes can be linked to exocrine pancreatic insufficiency (EPI). We evaluated the efficacy of the combined extracts with that of the antidiabetic drug glibenclamide in order to further investigate the anti-diabetic effects of *E. longifolia* root (*El*) and *Punica granatum* peels (*Pg*) on rats that had been streptozotocin (STZ)-induced to develop diabetics. One intraperitoneal dosage of streptozotocin (STZ, 60 mg/kg) caused the rats to experience hyperglycemia. Twenty-five Adult male Sprague Dawley divided into 5 equal groups (n = 5). The 1st group remained constant as a control.; 2nd group was normal rats were treated with combined extracts 200 mg/kg of *El:Pg* at ratio of (2:1); 3rd group was left untreated as a positive diabetics control; 4th group were diabetic treated orally with glibenclamide; while 5th group were diabetic treated with combination extracts 200 mg/kg of *El:Pg* at ratio of (2:1) for 25 days in consecutively. Researchers found that the diabetic effects of STZ, particularly blood glucose level, were greatly ameliorated by the combination *El:Pg* (2:1) extracts. Researchers found that the diabetic effects of STZ, particularly blood glucose level, were greatly ameliorated by the combination *El:Pg* (2:1) extracts. Additionally, there was a marked improvement in the variety of pancreatic histological lesions caused by STZ-induced diabetes. When compared to the reference medicine, glibenclamide, a combination of plant extracts containing *El:Pg* (2:1) improves fasting blood glucose levels and histopathological harmful effects caused by diabetes. It could be advised for diabetic patients suffering from the early alteration of pancreatic β -cells functions.

KEY WORDS: Diabetes; *Eurycoma longifolia*; *Punica granatum*; Combined extracts; Streptozotocin; Endocrine pancreas.

INTRODUCTION

Diabetes mellitus has become a major global public health concern, with its prevalence among adults more than tripling over the past two decades. Older age, overweight and obesity, unhealthy diet, physical inactivity, and smoking increase the risk of developing type 2 diabetes (Abdulmunem, 2025; Sidahmed *et al.*, 2025). Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by an imbalance between the body's insulin production and its needs, or a resistance to the insulin that the body does produce. Nearly 828 million people were diabetic in 2022, a 630 million increase from 1990 levels. The most significant growth was observed in nations with low or medium incomes in Southeast Asia (like Malaysia), South Asia (like Pakistan), North Africa and the Middle East (like Egypt), and Latin America and the Caribbean (like Jamaica & Costa Rica) (NCD Risk Factor Collaboration, 2024). When insulin-

responsive tissues, which are responsible for safely storing and metabolizing glucose, are denied enough insulin from the pancreatic β -cells, type 2 diabetes is induced. Complex and multifactorial, type 2 diabetes is driven by both environmental and genetic risk factors (Krentz & Gloyn 2020). The immune defense system is known to decline in a chronic diabetic state, leading to serious damage of vital organs and tissues, and there is a correlation between diabetes and this immune system (Oguntibeju *et al.*, 2020). Common oral hypoglycemic agents are costly and have serious side effects WHO suggested the importance of examining new hypoglycemic agents from plant origin due to fewer side effects. More than 800 plants have been identified through ethnobotanical research as potential traditional treatments for hyperglycemia (Jandari *et al.*, 2020). The bioactive chemicals found in pomegranate (*Punica granatum* L.) have

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long been utilized in traditional medicine to treat a wide range of illnesses (Rouhi *et al.*, 2017). Pomegranates are rich in a variety of phytochemicals, including polyphenols, fatty acids, amino acids, tocopherols, sterols, terpenoids, and alkaloids. These phytochemicals have medicinal uses, including reducing inflammation, acting as antioxidants, lowering blood sugar, and resisting microbes (Li *et al.*, 2006; Punasiya *et al.*, 2010; Grabez *et al.*, 2020; Osama Abdulmunem *et al.*, 2020). *Eurycoma longifolia* Jack is a South-East Asian medicinal herb that goes by the name "Tongkat Ali." Traditional medicine has long made use of plant components with demonstrated medicinal properties, including those against malaria, aphrodisiac effects, diabetes, bacteria, and fever (Bhat & Karim, 2010). The main objective of the current research is to treat normal and Streptozotocin-induced diabetic rats for 25 days straight with a combined extract of *Eurycoma longifolia* root and *Punica granatum* peels at a therapeutic dose for humans to determine its effect on beta cell counts and blood glucose levels.

MATERIAL AND METHOD

Chemicals. Regarding the chemicals, they were produced from Sigma-Aldrich Chemical Co (USA). Citric acid, sodium phosphate, chloroform, ethanol, phosphate buffer, were used in the study. Streptozotocin was purchased from EMD Millipore Company, Germany (572201-1GM). Glibenclamide, normal saline, glucometer and glucometer test strips (ACCU-CHEK® Instant S) were purchased from a local pharmacy in Malaysia, Pahang, Kuantan.

Ethical issues. The Animal Ethics Approval under the IACUC Chairpersons/ University Malaysia Pahang, reference number (UMPIACUC/2018/02). Every step of the animal care process adhered to the guidelines laid out by the National Institutes of Health's (NIH) Guide for the Care and Use of Laboratory Animals. (National Institutes of Health, 1985).

Ultrasound-assisted extraction of plants. *E. longifolia* (El) and *P. granatum* (Pg) were procured from a dealer (Kedai Ubat Aw Kah Yan Sdn. Bhd) in a popular market in Kuantan, Pahang, Malaysia. Before being ground into powder, the plants were rinsed with distilled water after being washed in running water. After seven days of drying at room temperature, they were ground using PULVERISETTE 19 Cutting Mill System (P19) 15.4020/02399 (grinding size 1mm) and kept in sealed plastic jars. To summarize, 50 g of each plant material was placed on a beaker. For the *Eurycoma longifolia* and *Punica granatum* extractions, 400 mL of ethanol (EtOH) and 400 mL of chloroform, respectively, were used. The sonicator's ultrasonic probe was submerged in the sample-containing beaker and subjected to 80 W of ultrasound power. A 45 °C ultrasonic

bath has been used to regulate the extraction process's temperature. At intervals ranging from 1 to 180 min, each batch underwent an extraction process that continued for 15 min. An EYELA N-1200 Series rotary vacuum evaporator was used to concentrate the filtrate at the end. A 48-hour air-drying process was followed by preservation at -20 °C for future usage of the obtained concentrates (Sivakumar *et al.* 2011).

Acute toxicity test. The safety profile of the *El:Pg* (2:1) combined extracts was determined by the procedure defined in the revised procedure, Method for UP and Down (Organization for Economic Cooperation and Development, 2008). In short, a total of six male Sprague Dawley rats (one rat per day) were used for the test. The rats were fasted overnight (16 h) and each rat's body weight (g) was registered prior to the test. A fixed dose of *El:Pg* (2:1) combined extracts (5 g/kg) was administered orally to each rat and closely examined at 4 h initially, then every 6 h intervals for behavioral changes (alertness, restlessness, irritability, recumbency, vomiting, anxiety), neurological changes (spontaneous movement, convulsions, gait, orifices of bleeding, touch/pain reaction), autonomic changes in behavior, (defecation, micturition) and/or mortality profiles (Yankuzo *et al.*, 2011). The extract volume was changed to contain 600 mg/ml and any significant morbidity or mortality within (24-72 h) has been registered.

Experimental animals. In this study Sprague Dawley rats (males, 8-10 weeks old, 158-167 g) were used in this experiment and were purchased from A SAPPHIRE ENTERPRISE (001303794- M), Selangor, Malaysia. Initially, the animals were brought up in conventional laboratory settings by being housed in polypropylene cages. These conditions included a temperature of 24 ± 2 °C, sufficient cross ventilation, and a 12-hour light/dark cycle (from 7:00 AM to 7:00 PM).; and relative humidity = 46-79 %. The rats were fed with standard commercial dry pellets that contain crude Protein (22 %), Fat (46 %), Ash (7.6 %), Moisture (12 %), Fiber (4 %), Calcium (1.2 %), and Phosphorus (0.73 %) (Gold Coin Feed Mills Sdn. Bhd. Kuala Lumpur, Malaysia).

Induction of diabetes. Before being given diabetes, the rats fasted for 12 to 14 h at night, with just water accessible to them. The following procedure was to inject 60 mg of streptozotocin (STZ) per kilogram of body weight into the animals' intraperitoneal spaces. Prior to getting the injection, the drug (STZ) was reconstructed in freshly prepared 0.1 M citrate buffer (pH 4.5) and injected immediately to avoid degradation. After STZ injection, Experimental animals were given 1 mL of glucose solution (5 % w/v) 6 h after injection instead of tap water to starve off hypoglycemic shock. The

rats' fasting blood glucose (FBG) levels were measured using an Accu-Chek Instant S Glucometer (Roche, Mannheim, Germany) five days after induction. Only rats that exhibited hyperglycemic symptoms (FBG > 14 mmol/L) were selected for later experiments (Erejuwa *et al.*, 2011; Balogun & Ashafa, 2017).

Experimental design. The rats were divided into 5 groups (n=5). The fasting blood glucose levels were estimated before starting the experiment. The groupings and treatments are as follows:

- Group 1: Normal rats received 0.01 % carboxymethyl cellulose (CMC), by oral gavage daily for 25 days, used as a normal control (NC).
- Group 2: Normal rats were orally given 200 mg/kg of *El:Pg* (2:1) diluted with 0.01 % CMC once daily for 25 days (*NEl:Pg*).
- Group 3: Diabetic controls were used as a positive control; 1 % CMC was given via oral gavage once daily for 25 days (DC).
- Group 4: Diabetic rats treated with the *El:Pg* (2:1) extracts (200 mg/kg BW/day dissolved in 1 % CMC) by oral gavage once daily for 25 days (*DEl:Pg*).
- Group 5: Diabetic rats treated with 0.6 mg/kg of glibenclamide by oral gavage once daily for 25 days.

Treatments started on day 6 after STZ injection and were regarded day 1 for treatment and sustained for 25 days.

Weight measurement. The rats were subjected to body weight measurement (g) on the last day of the experiment. The body weights were measured using a Mettler Toledo SB16000 Balance (Painesville, OH, USA).

Measurement of fasting blood glucose. The fasting blood glucose was monitored on day 5 and 30. The blood glucose level was measured by making tiny puncture at the tip of the tail using Accu-Chek Lancets. The distal area of the tail was swabbed with alcohol (ethanol 70 %), and one drop of blood was withdrawn. An Accu-Chek Performa glucometer was used to measure the level of fasting blood glucose.

Histopathology study. On the thirtieth day of the study, ketamine and xylaxin were used as an anesthetic drug for sacrificing rats. Pancreas from each sacrificed rat was taken away after using perfusion fixation technique and put directly in a labeled container filled with 10 % formalin for 72 h as a fixative. After 72 h, the pancreas was washed with normal sailing for about 20-30 min and preserved in 70 % ethanol till later histopathological examination (Yahya *et al.*, 2013). The histopathological evaluations were done at Kulliyyah of Pharmacy, Department of Basic Medical Science, IIUM, Kuantan.

Statistical analysis. The analysis of the experimental data was done using IBM SPSS Statistics 24 (IBM Corporation, NY, USA). The results of the analysis were presented as mean $\pm$ standard deviation (SD) of the analyzed datasets. Test of significance was performed at p-value < 0.05; intra-group data were compared using Student t-test.

RESULTS

Acute toxicity test of *El:Pg* (2:1) combined extract. The non-toxic safe essence of *El:Pg* (2:1) combined extract was discovered in an acute toxicity analysis. At a single large dose of 5000 mg/kg B.W., no evidence of toxic effects or major behavioral changes was found in all animals. Similarly, within 24 h of observation and later, no mortality was reported (Table I).

Fasting blood glucose level. Statistically, there were significant differences (p<0.05) in fasting blood glucose levels between diabetic control group and normal control. The changes in fasting blood glucose levels in diabetic group treated with *El:Pg* (2:1) were statistically significant in comparison with the diabetic control group (p<0.05) as shown in Figure 1.

Effects of extracts combinations on body weights. The reduction in body weight in the diabetes control group was significant relative to the normal control group (p <0.05). The diabetic group of rats treated with *El:Pg* (2:1) exhibited no significant difference in mean body weight compared to the diabetic control group (p>0.05), as shown in Figure 2.

Table I. Data on safety profile estimation in rats after treatment with *El:Pg* (2:1) combined extracts (5000 mg/kg).

Body weight (g)	Dosage (mg/kg)	Physical activity	CNS Toxicity	ANS Profile	Mortality
170	850.00	-	-	-	-
174	870.00	-	-	-	-
177	885.00	-	-	-	-
160	800.00	-	-	-	-
175	875.00	-	-	-	-
171	855.00	-	-	-	-

-, absence of death/significant activity; CNS, central nervous system; ANS, autonomic nervous system.

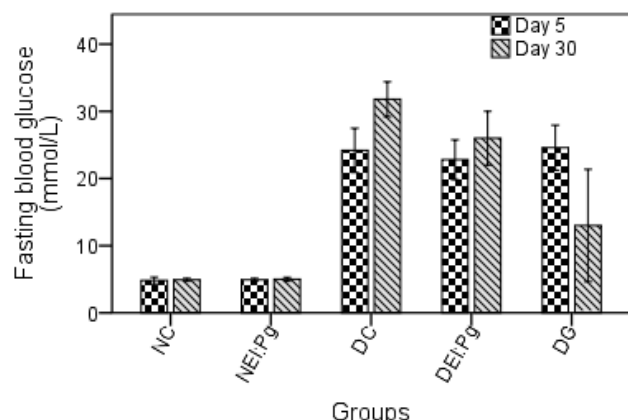


Fig. 1. Effects of *El:Pg* (2:1) treatment on fasting blood glucose levels (mmol/L) in STZ-induced diabetic rats relative to the conventional medication Glibenclamide. $p < 0.05$ for both control and treated groups; values are expressed as mean $\pm$ SD; $n = 5$; error bar represents standard deviation.

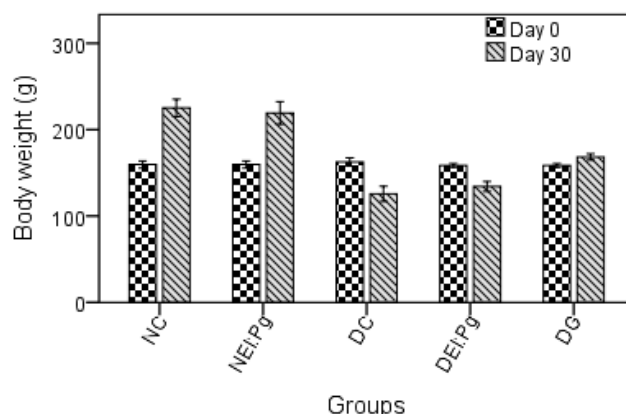


Fig. 2. Effect of 25 days administration of *El:Pg* (2:1) on body weight of normal and STZ-diabetic rats. Each value is the mean $\pm$ SD, $n = 5$.

Histopathological analysis. As reflected in Figure 3, pancreas of diabetic control rats has shrunken pancreatic islets (islets of Langerhans) with scanty number of small sized pancreatic islets scattered within the acini. On the contrary, diabetic rats treated with glibenclamide and *El:Pg* (2:1) combined extracts showed exhibited less pathological changes and mild improvement in their structure with restored increase in the number and size of pancreatic islets.

DISCUSSION

High blood glucose levels, insulin resistance, and relative insulin insufficiency characterize type 2 diabetes mellitus, the most prevalent type of DM (Atlas 2017). Furthermore, by using relevant animal models for research, we have been able to better understand the molecular mechanisms that underline the onset, progression, and therapeutic management of diabetes, as well as its metabolic, genetic, and environmental risk factors (Abdel Aziz *et al.*, 2020). In this investigation, the antidiabetic effect of *El:Pg* (2:1) combined extracts was investigated using the STZ diabetic rat model, which is characterized by abnormalities in blood glucose level, body weight, and pancreatic histology. Several investigations have shown that the red color of the *Punica granatum* peel is caused by chemicals with antidiabetic potential, such as anthocyanidins, flavanols, flavones, flavanones, punicalin, and punicalagin (Bharti *et al.*, 2018; Ramya *et al.*, 2020). *Eurycoma longifolia* extracts contained 100 % glycoside, phenolic and tannin compounds 80 %, steroid 60 %, flavonoids and alkaloids 20 % (Mahmood *et al.*, 2019). The findings of the acute toxicity test (Table I) showed a high safety margin and an absence of single-dose mortality

of up to 5000 mg/kg of body weight. In addition, the minimum lethal dose (LD50) could not be calculated. The sub-chronic toxicity of the standardized aqueous *E. longifolia* extract (Physta®) in Wistar rats was determined to be 1000 mg / kg (the appropriate daily intake [ADI] for adults is 600 mg / day) with no reported adverse reactions (NOAEL). Pomegranate peel ethanol extract has been classified as safe based on aquatic toxicity classification by the Organization for Economic Cooperation and Development (OECD) (2008). Previous reports on the power of STZ to induce diabetes in experimental rats have been confirmed by the considerable increase of fasting blood glucose level in the diabetic untreated rat group. Elevation of blood glucose levels caused by selective destruction of β -cells of the pancreatic islets in the pancreas by the effect of STZ primarily results in decreased glucose uptake by muscle and adipose tissue, increased glycogen breakdown, and increased gluconeogenesis and hepatic glucose production (Abu Abeelee *et al.*, 2009; Deeds *et al.*, 2011; Nagy *et al.*, 2012). Diabetes rats had a reduction in fasting blood glucose levels after being orally administered *El:Pg* (2:1) extracts (Fig. 1). The current findings are consistent with earlier reports of the antidiabetic effects of *El:Pg* (2:1) extracts, which are mediated by the main component, secoisolariciresinol diglucoside (SDG), which inhibits hepatic gluconeogenesis through its antioxidant activity and suppression of phosphoenolpyruvate carboxykinase (PEPCK) gene expression (Prasad, 2002; Pan *et al.*, 2007). The effect of oral glibenclamide on FBG was found to be more effective than *El:Pg* (2:1) extracts (Fig. 1). Our previous study in vitro, *El:Pg* (2:1) combination extracts have inhibitory activity against α -amylase and α -glucosidase, which is efficient in glucose uptake (Osama Abdulmunem *et al.*,

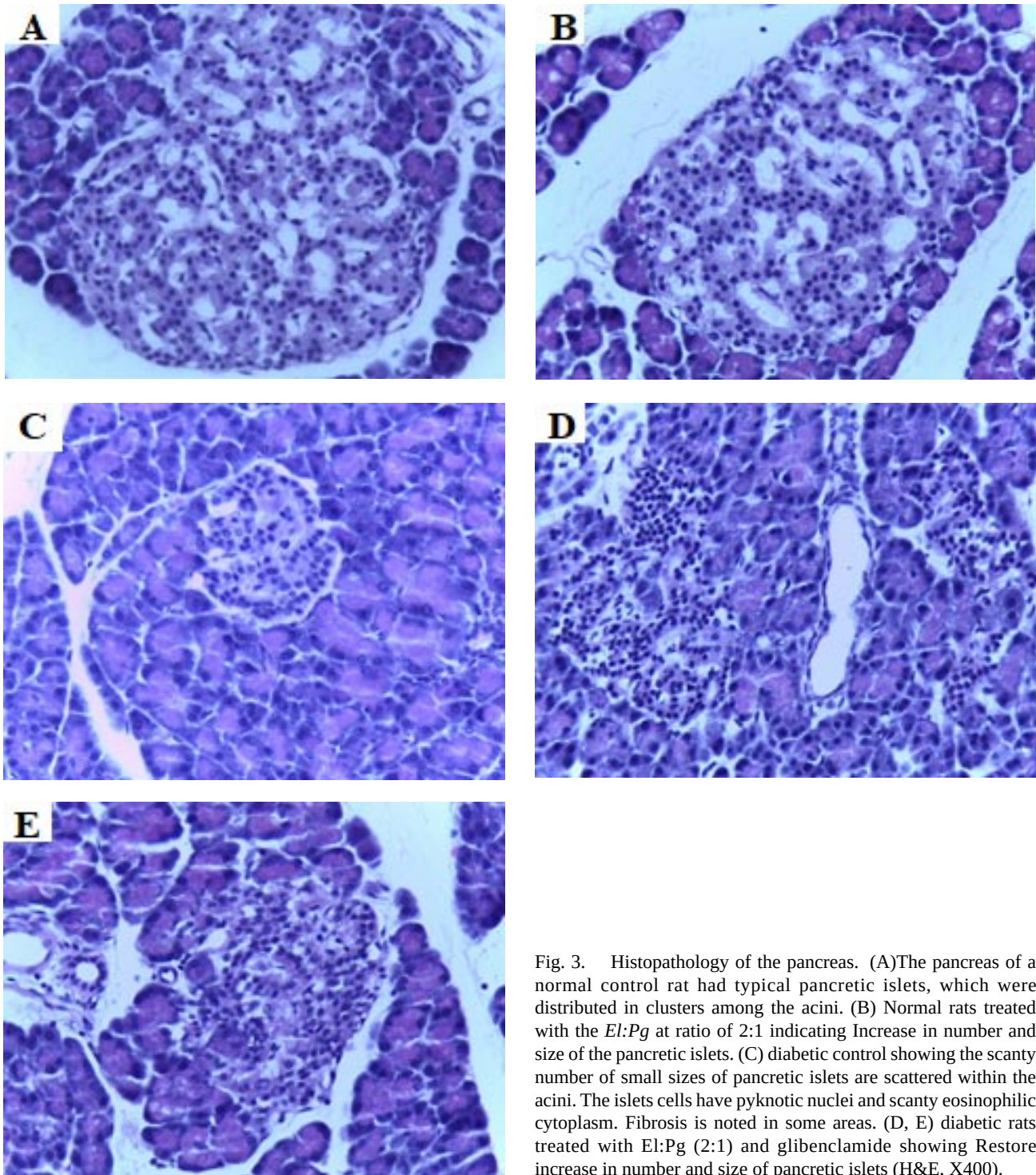


Fig. 3. Histopathology of the pancreas. (A) The pancreas of a normal control rat had typical pancreatic islets, which were distributed in clusters among the acini. (B) Normal rats treated with the *El:Pg* at ratio of 2:1 indicating Increase in number and size of the pancreatic islets. (C) diabetic control showing the scanty number of small sizes of pancreatic islets are scattered within the acini. The islets cells have pyknotic nuclei and scanty eosinophilic cytoplasm. Fibrosis is noted in some areas. (D, E) diabetic rats treated with *El:Pg* (2:1) and glibenclamide showing Restore increase in number and size of pancreatic islets (H&E, X400).

2020). The primary phenolic chemicals oversee decreasing oxidative stress, lipid peroxidation, suppression of α -glucosidase, and improved glucose absorption (Hossain *et al.*, 2020). In our previous study, the outcome of the study suggests that the *Punica granatum* peel and *Eurycoma*

longifolia roots extracts have the potential to be used as antioxidant (Mahmood *et al.*, 2019). The presence of phenolic compounds, alkaloids, terpenoids and flavonoids can be responsible for the antidiabetic function of medicinal plants (Patle *et al.*, 2020).

The STZ-induced diabetic rats in this study showed severe body weight loss due to structural proteins degradation during DM (Ramesh & Pugalendi, 2006; Salahuddin *et al.*, 2010; Zafar & Naeem-Ul-Hassan Naqvi, 2010; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee & Cheng, 2013; Zari & Al-Thebaiti, 2018). Gluconeogenesis, which is linked to increased muscle wasting and protein loss in tissues, is another possible explanation for the weight loss in STZ diabetic rats (Patel *et al.*, 2014). In this study, the STZ-induced diabetic rats revealed a severe loss of body weight which was started in the second week. This loss of body weight was related to the structural proteins degradation which has a vital effect on the body weight (Kalaivanan & Pugalendi, 2011). Oral administration of *El:Pg* (2:1) extracts and glibenclamide reversed the changes in the body weight in the diabetic rats, these changes occur due to the ability of these treatments to decrease hyperglycemia (Fig. 2). In the present study, the enhancement of determined parameters we observed during the last days of the experiment. The therapeutic effect of the long-term use of *El:Pg* (2:1) extracts could be tested by extending the experiment duration for a few more weeks. The ability of certain herbal extracts to treat diabetes and prevent onset of DM-related long-term complications with no serious adverse effects has been reported. For instance, the antidiabetic property of *E. longifolia* and *P. granatum* has been reported by several researchers (Khajebishak *et al.*, 2019; Loizzo *et al.*, 2019; Talbott, 2019; El Deeb *et al.*, 2021). Various histological alterations were observed in pancreatic sections of rats subjected to streptozotocin-induced diabetes. These included a reduced islet of Langerhans with degranulated and degraded b-cells, vacuolations, hyaline deposits, and clogged capillaries. This might provide light on the mechanism of hepatoprotective effect of *El:Pg* (2:1) extracts by revealing their capacity to scavenge free radicals produced during diabetes. These finding are in agreement with many previous studies on the action of STZ on endocrine pancreas (Rifaai *et al.*, 2012; Motshakeri *et al.*, 2014; Kazeem *et al.*, 2015). However, less pathological changes and mild improvement with restoration in the structure of Pancretic islets were seen in *El:Pg* (2:1) extracts and glibenclamide-treated diabetic rats; these changes are in parallel with previous reports on the effects of these treatments on the endocrine pancreas which indicate the preventive effects of these treatments against the damage caused by diabetes (Banihani *et al.*, 2013; Ahmed *et al.*, 2014; Rehman *et al.*, 2016).

CONCLUSION

Potentially, the glycemic status in STZ-induced diabetic rats can be improved by increasing insulin sensitivity in peripheral tissues and improving the secretory response

of β -cells when *El:Pg* (2:1) extracts are administered orally. There is hope for a cure in the form of antidiabetic and pancreatic protecting effects from *El:Pg* (2:1) extracts. It follows that *El:Pg* (2:1) extracts and their active ingredients merit additional research into their potential as a safe and effective medicinal treatment for type 2 diabetes mellitus and its pathological effects.

ACKNOWLEDGMENTS. I extend deep thanks to Al-bayan University for their great financial and scientific support.

ABDULMUNEM, K. O. & AUNG, S. Efectos pancreáticos de los extractos combinados de *Eurycoma longifolia* y *Punica granatum* en la diabetes inducida por estreptozotocina en ratas. *Int. J. Morphol.*, 44(4):1090-1097, 2026.

RESUMEN: La diabetes y la obesidad son dos trastornos de salud pública debilitantes que frecuentemente se presentan juntos y ambos están en aumento. A pesar de ello, la diabetes se ha convertido en una epidemia mundial. Todas las formas de diabetes pueden estar relacionadas con la insuficiencia pancreática exocrina (IPE). Evaluamos la eficacia de los extractos combinados con la del fármaco antidiabético glibenclamida para investigar más a fondo los efectos antidiabéticos de la raíz de *E. longifolia* (El) y las cáscaras de *Punica granatum* (Pg) en ratas a las que se les había inducido la diabetes con estreptozotocina (STZ). Una dosis intraperitoneal de estreptozotocina (STZ, 60 mg/kg) provocó que las ratas experimentaran hiperglucemia. Veinticinco machos adultos Sprague Dawley se dividieron en 5 grupos iguales (n = 5). El primer grupo permaneció constante como control; el segundo grupo de ratas normales fueron tratadas con extractos combinados de 200 mg/kg de *El:Pg* en una proporción de (2:1); el tercer grupo se dejó sin tratamiento como control positivo para diabéticos; el cuarto grupo las ratas fueron tratadas por vía oral con glibenclamida para diabéticos; mientras que el quinto grupo las ratas fueron tratadas con extractos combinados de 200 mg/kg de *El:Pg* en una proporción de (2:1) durante 25 días consecutivos. Los investigadores encontraron que los efectos diabéticos de STZ, particularmente el nivel de glucosa en sangre, fueron mejorados en gran medida por la combinación de extractos *El:Pg* (2:1). Además se descubrió que los efectos diabéticos del STZ, en particular la glucemia, se vieron significativamente mejorados por la combinación de extractos *El:Pg* (2:1). Además, se observó una marcada mejoría en la variedad de lesiones histológicas pancreáticas causadas por la diabetes inducida por STZ. En comparación con el medicamento de referencia, la glibenclamida, una combinación de extractos vegetales que contiene *El:Pg* (2:1) mejora la glucemia en ayunas y los efectos histopatológicos nocivos causados por la diabetes. Podría recomendarse para pacientes diabéticos que sufren una alteración temprana de la función de las células β pancreáticas.

PALABRAS CLAVE: Diabetes; *Eurycoma longifolia*; *Punica granatum*; Extractos combinados; Estreptozotocina; Páncreas endocrino.

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