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Hepatoprotective Effects of Combined Extracts of *Eurycoma longifolia* and *Punica granatum* in Streptozotocin-Induced Diabetic Rats

Efectos Hepatoprotectores de Extractos Combinados de *Eurycoma longifolia* y *Punica granatum* en Ratas con Diabetes Inducida por Estreptozotocina

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SUMMARY: Diabetes mellitus (DM) is considered a metabolic syndrome of many etiologies that is treated currently with many brands of prescribed drugs, each of which has its own set of negative effects. As such, natural alternatives from medical plants are being explored as remedies for treating DM owing to their little or no negative effects on the body. In this work, the effects of combining chloroform peel extract of *Punica granatum* (*Pg*) and ethanolic root extract of *Eurycoma longifolia* (*El*) at a combined ratio of 2:1 on liver function in streptozotocin (STZ)-induced diabetes rats was studied. The Sprague Dawley adult male rats were split into 4 groups (n = 5); The rats in Group 1 were maintained as the normal control; the rats in Group 2 were left untreated following administration of STZ (positive diabetic control); the rats in Group 3 received oral glibenclamide treatment for diabetes following injection of STZ; and the rats in Group 4 received the combined extracts (200 mg/kg of *El: Pg* at a 2:1 ratio) for 25 consecutive days. The levels of blood glucose and the weights of the body and liver of the rats in each group were measured at the end of the study period. A histological analysis of the liver and an investigation of liver function were conducted. In diabetic rats, the combined *El:Pg* (2:1) extracts increased the rate of weight loss and levels of fasting blood glucose (p 0.05); the diabetic rats also had reduced liver enzyme (AST, ALP, ALT, and GGT) levels compared to the diabetic control rats ($p < 0.05$). The histological study showed sinusoidal congestion, severe to moderate vacuolation in the cytoplasm of the liver cells, and minor disarrangement of the hepatic cord in the rats treated with *El:Pg* combined extract. This study experimentally demonstrated the antihyperglycemic properties of *El:Pg* (2:1) that may be useful in halting the progression of diabetics via hepatoprotective actions.

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

INTRODUCTION

One of the biggest contributors to international health crises in the twenty-first century is still diabetes. One in eleven persons, according to the International Diabetes Federation (IDF), has diabetes. More than 463 million people have diabetes diagnoses in 2019, and it's expected the figure would rise to 578 million by 2030 and 700 million by 2045 (International Diabetes Federation, 2013; Ogurtsova *et al*., 2017). Diabetes mellitus (DM) results from the inability of the body to secret insulin, a hormone responsible for sugar control in the body, which significantly affects the metabolism of lipids, carbohydrates, and proteins (American Diabetes Association, 2017; Landon *et al*., 2020). Among the long-term impacts of DM are inflammation, macrovascular and microvascular abnormalities, cirrhosis, and apoptosis; it also "causes liver injury (Su *et al*., 2020). In clinical practice, liver function tests are frequently used to identify and treat liver problems. The integrity of hepatocytes is typically indicated by liver enzymes such as aspartate aminotransferase (ASAT; EC 2.6.1.1), alanine aminotransferase (ALAT; EC 2.6.1.2), gamma-glutamyl aminotransferase (GGT; EC 2.3.2.2), and albumin (Harris, 2005)".

According to studies, the odds of a damaged liver healing during the acute stage are reduced, which results in a chronic condition with problems. Despite tremendous advancements in medicine, only a few available drugs can both protect the liver from harm and boost liver functioning. Hence, many people with liver disease employ herbal treatments to treat their medical conditions (Parmar *et al*., 2010). According to estimates, traditional medicines are used by 80 % of the world's population for primary healthcare because they are widely available, affordable, safe, and effective (World Health Organization, 2013). Several plants have historically been useful for treating diabetes, and many

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more are currently being researched for their potential use in the treatment of diabetes. These herbal treatments are thought to be capable of treating the illness and its negative consequences on the cells of the body (Modak *et al*., 2007; Kesari *et al*., 2007).

E. longifolia, known locally as *tongkat ali* in Malaysia, *tung saw* in Thailand, *pasak bumi* in Indonesia, and *cay ba bihn* in Vietnam is one of the strong medicinal plants from the Simaroubaceae family (Thu *et al*., 2018). The extracts of *E. longifolia* have been shown to exhibit many biological functions, such as anti-osteoporotic, anti-malarial, antibacterial, antioxidant, anti-cancer, anti-proliferative, anti-inflammatory, aphrodisiac, anti-rheumatism, anti-diabetic, anti-ulcer, and anxiolytic activities (Thu *et al*., 2018). Most of the phytochemical contents of *E. longifolia*, such as quassinoids, terpenes, glycoproteins, polyphenols, alkaloids, and high molecular weight polysaccharides are found in the roots (Tsai *et al*., 2020). Another plant, pomegranate (*Punica granatum* L.) is native to the Middle East but was introduced to North America in the 18th century by the Spanish settlers (Fischer *et al*., 2011). The juice and fruit of pomegranate are said to exhibit anti-hyperglycemic and anti-diabetic properties (Khalil, 2004; Punasiya *et al*., 2010). The combination of different plant extracts rather than using individual plant extracts, has been a normal traditional practice in many circumstances of Indian medicinal plant compositions (Kavitha, 2019). The prospect of a therapeutic interaction when used in conjunction with a traditional antidiabetic drug, either purposefully or unintentionally, is suggested by the fact that the plant has antidiabetic qualities and is also frequently used for edible purposes. Being that the interaction between the components of different plants could be additive, antagonistic, or synergistic, it is necessary to conduct a systematic investigation of the impacts of such combinations before approval for human use via animal studies (Michael *et al*., 2010).

Until now, studies are yet to report the effects of combined extracts of the roots of *El* and the peels of *Pg* for the control of hyperglycemia-related hepatotoxicity. It is expected in this study that the combined extracts of the root of *El* and peels of *Pg* will reduce the development and progression of hyperglycemia-induced hepatic damage in laboratory animals, in addition to its known antihyperglycemic activities.

MATERIAL AND METHOD

Chemicals and reagents. The employed plant materials for this study were purchased from a vendor in a well-known market in "Malaysia (Table I) and processed in the laboratory unit of the Faculty of Industrial Sciences & Technology (FIST), University Malaysia Pahang, Malaysia. The materials were washed thoroughly, pulverized, and stored in airtight bags for further use. The other reagents for the study were purchased from Sigma-Aldrich Chemical Co. (USA)." Streptozotocin (572201-1GM) was "supplied by EMD Millipore, Germany while glibenclamide and other materials like normal saline, glucometers, and glucometer test strips (ACCU-CHEK® Instant S) for the hyperglycemia tests were purchased from a registered pharmacy in" Malaysia.

Ultrasound-Assisted extraction of plant materials. The extraction of the plant materials was done using a method described by Sivakumar *et al*. (2011); 50 g of each plant material was introduced into a beaker; *P. granatum* was "extracted with 400 mL of chloroform (Chl) while *E. longifolia* was extracted with 400 mL of ethanol (EtOH). The ultrasonic probe of a sonicator was inserted into the beaker containing the samples to begin the extraction procedure while operating at an ultrasound power of 80 W. The procedure was kept at a 45 °C temperature and the sonication bath was sonicated for 15 min at intervals of 1 to 180 min. The samples were filtered, and the filtrate was concentrated in a rotating vacuum evaporator (Eyela N-1200 Series). The concentrated filtrate was dried for 48 h at room temperature and preserved at -20 °C for analysis."

Acute toxicity test. The method specified in the updated procedure, Procedure for UP and Down, was used to determine "the safety profile of the *El:Pg* (2:1) mixed extracts (The Organisation for Economic Cooperation and Development OECD, 2008). Briefly, six male Sprague Dawley rats one each day were employed for the test. Before the experiment, each rat's body weight (g) was recorded after an overnight fast (16 h). Each rat received a fixed dose of the combined *El:Pg* (2:1) extracts (5 g/kg), which were then closely monitored for behavioral, neurological, autonomic, and/or mortality changes at intervals of 4 h, 6 h, and 12 h. The monitored behavioral changes included alertness, recumbency, vomiting, restlessness, irritability, and anxiety; neurological changes

Table I. Nomenclature and parts of the plant materials used in this study.

No.	English name	Scientific name	Code	Family	Part used
1	Longjack	*Eurycoma longifolia*	*El*	Simaroubaceae	Roots
2	Pomegranate	*Punica granatum*	*Pg*	Lythraceae	Peels

include convulsions, gait, spontaneous movement, orifices of bleeding, and touch/pain reaction; while autonomic changes in behavior include defecation and micturition; the mortality profiles were also monitored (Yankuzo *et al*., 2011). The volume of the extract was changed to 600 mg/ml if any significant morbidity or fatality is noticed within 24-72 h of" administration of a higher dose.

Animal groups and the treatments. This study involved 25 mature male Sprague Dawley rats weighing between 158 and 167 grams and being "8 to 10 weeks old; the rats were purchased from A SAPPHIRE ENTERPRISE (001303794- M), Selangor, Malaysia. First, the rats were acclimated by keeping them in polypropylene cages with conventional laboratory settings (relative humidity = 46-79 %, temperature = 24 2 °C, adequate cross ventilation, and 12-hour light/dark cycle (7:00 AM to 7:00 PM). The rats were fed with standard dry commercial pellets that contain 22 % crude protein, 46 % fat, 7.6 % ash, 12 % moisture, 4 % fiber, 1.2 % calcium, and 0.73 % phosphorus;" the pellets were purchased from Gold Coin Feed Mills Sdn. Bhd. Kuala Lumpur, Malaysia. The National Institutes of Health's guidelines and standards for the Care and Use of Laboratory Animals, as well as the Animal Ethics Clearance issued by the IACUC Chairpersons, with the University Malaysia Pahang, reference number (UMPIACUC/2018/02), were observed while handling the animals.

Induction of diabetes. Before inducing diabetes, the rats were starved for 12 to 14 h; streptozotocin (STZ) was then administered intraperitoneally to the animals at a dose of 60 mg/kg body weight. To prevent degradation, STZ was resuspended in freshly made 0.1 M citrate buffer (pH 4.5) before being administered. And to prevent hypoglycemia shock after STZ injection, 1 ml of 5 % w/v glucose solution was administered to the test animals instead of tap water six hours later. The rats' fasting blood glucose (FBG) level was measured using an Accu-Chek Instant S Glucometer (Roche, Mannheim, Germany) five days after induction. For subsequent tests, only rats with hyperglycemic symptoms (FBG > 14 mmol/L) were used (Erejuwa *et al*., 2011).

Experimental design and protocol. There were 4 groups of rats (n=5). Before starting the study, the FBG levels were estimated.

Group 1: These are normal rats that served as the negative control; they were exposed to 0.01 % carboxymethyl cellulose (CMC) via oral gavage every day for 25 days.

Group 2: Rats with diabetes which served as the positive control; the rats in this group received 1 % CMC orally once daily for 25 days.

Group 3: These are diabetic rats that received oral gavage treatments of the *El:Pg* (2:1) extracts (200 mg/kg BW/day diluted in 1 % CMC) for 25 days.

Group 4: These are diabetic rats given 0.6 mg/kg glibenclamide as a treatment for DM.

Treatments began on the sixth day following STZ injection, and were considered Day 1 for treatment, and continued for 25 days.

Body and liver weight measurement. The body weight (g) of the rats was measured "using a Mettler Toledo SB16000 Balance. Equation 1 was used to calculate the relative liver weights (RLW) of the sacrificed rats."

$$\text{RLW \%} = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

Biochemical analysis. To check the levels of blood glucose of the animals, Accu-Chek Lancets were used to make tiny punctures near the rat tail's tip while Accu-Chek Performa glucometer was used to determine the blood glucose level. Blood for renal function tests was drawn from the rat's orbital sinus under minimal anesthesia using ketamine and xylazine. The tests for liver function were performed at the Gribbles Pathology Laboratory, Kuantan, Malaysia; the liver function profile tests include tests for serum enzymes (ALT, AST, GGT, ALP), and tests for serum proteins (total protein, albumin, and globulin).

Histopathology Study. Ketamine and xylazine were employed as anesthetic drugs to sacrifice the rats on the thirty-first day of the study. The histopathology analyses were completed at the "Kulliyyah of Pharmacy, Department of Basic Medical Science, International Islamic University Malaysia (IIUM), Kuantan." Each sacrificed rat's liver, pancreas, and both kidneys were removed after utilizing the perfusion "fixation procedure and placed directly in a marked container filled with 10 % formalin for 72 hours to serve as a fixative. Following 72 hours, the organs were" stored in 70 % ethanol until a subsequent histological investigation after being rinsed with normal saline for 20 to 30 minutes (Yahya *et al*., 2013).

Statistical analysis. IBM SPSS Statistics 24 (IBM Corporation, NY, USA) was used to analyze the experimental data. The outcome of the analysis was displayed as the mean ± standard deviation (SD) of the datasets. Data within groups were compared using the student t-test, and the test of significance was run at a p-value of 0.05.

RESULTS AND DISCUSSION

Percentage yield of plant extracts. The yield of the extracts from the 50 g of extracted plant materials was 5.7 % for *Punica granatum* and 6.0 % for *Eurycoma longifolia*.

Acute toxicity test of combined *El:Pg* extracts. The acute toxicity investigation revealed that the combined El: Pg extracts at the ratio of 2:1 was safe and non-toxic as all the animals that received a single high dose of 5000 mg/kg B.W of the extracts combination showed no signs of toxicity or significant behavioral abnormalities; furthermore, no death was documented within 24 hours of monitoring (Table II).

The "acute toxicity test results (Table II) revealed a high safety margin and no single-dose fatality at doses up to 5000 mg/kg body weight. it was impossible to determine the minimum lethal dose (LD50). Choudhary *et al*. (2012), determined that the sub-chronic toxicity of the standardized aqueous *E. longifolia* extract (Physta ®) in Wistar rats was 1000 mg/kg but in adults, the approximate daily intake [ADI] is 600 mg/day with no adverse reactions (NOAEL)." According to aquatic toxicity categorization, the Organization for Economic Co-operation and Development (OECD) has rated the ethanol peel extract of pomegranate as safe (Wibowo *et al*., 2018).

Evidence of Diabetes

Fasting blood glucose. On the fifth day, the mean FBG level in the DC group was 24 ± 1.64 mmol/L, but on the tenth day, it had risen to 28 ± 1.10 mmol/L and steadily increased until it reached 32 ± 1.30 mmol/L on the thirty-first day. The mean FBG level in the DE*l:Pg*C (2:1) group was found to be 23 ± 1.48 mmol/L on the fifth day which progressively increased until day 10 when it peaked at 27 ± 1.30 mmol/L; then, there was a slight decrease on day 20 before increasing once again to 26 ± 2.00 mmol/L on last day of the study. The observed "changes in the FBG levels of the DE*l:PgC* (2:1) group (in comparison to the DC group) were statistically significant at $p < 0.05$ (Fig. 1). The differences in the FBG levels of the *El:Pg* (2:1) and glibenclamide-treated groups were statistically significant when compared" to the DC group ($p < 0.05$).

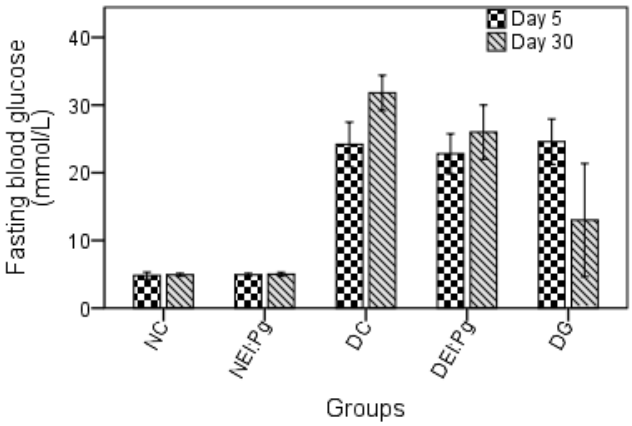

Fig. 1. Effect of El:Pg (2:1) administration on FBG level of different animal groups after 25 days of administration compared to the control groups (NC = normal control; DC = diabetic control, DEl: Pg = diabetic group treated with extract combination; DG = diabetic group treated with glibenclamide.

A metabolic disorder with multiple etiologies, diabetes mellitus (DM) presents as hyperglycemia primarily as a result of the body's complete failure to produce insulin in response to elevated serum sugar levels or as a result of the body's cells' inability to react to the presence of insulin (Letchuman *et al*., 2010). One STZ injection (60 mg/kg I.P.) was used to cause diabetes by partially destroying pancreatic b-cells of the pancreatic islets of Langerhans, this results in decreased absorption of glucose by adipose tissues and muscles, coupled with increased gluconeogenesis, the elevation of glycogen breakdown, and increased glucose synthesis by the liver (Abeeleh *et al*., 2009; Deeds *et al*., 2011; Aboonabi *et al*., 2014; Adams & Yakubu, 2020).

The *El:Pg* plant extracts were given to STZ-induced diabetic rats to reduce their elevated FBG levels (Fig. 1). The decreased FBG levels suggest that *El:Pg* extracts could have an effect on peripheral tissues similar to that of insulin

Table II. The safety profile of rats treated with combined *El:Pg* extracts (5000 mg/kg) at the combination ratio of 2:1.

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	-	-	-	-

Note: - = No death/observable activity; ANS = autonomic nervous system; CNS = central nervous system.

by either boosting glucose absorption metabolism or blocking glucose synthesis by the liver (Eliza *et al*., 2009). In diabetic rats, oral treatment with *El:Pg* (2:1) plant extracts reduced FBG levels which may also promote beta islet insulin secretion and improved insulin sensitivity to glucose uptake. The current findings are consistent with earlier reports of the anti-diabetic properties of *El:Pg* plant extracts which are mediated by the antioxidant activity of secoisolariciresinol diglucoside (SDG), the major component, and by inhibition of hepatic gluconeogenesis via the suppression of the expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene (Prasad, 2002; Pan *et al*., 2007).

Animal weights. Rats with diabetes had considerably ($p < 0.05$) lower body weights than normal controls (Fig. 2). The rate of observed weight loss in untreated DC rats appears to be slowed down by treatment with glibenclamide and *El:Pg* extracts. In comparison to the DC group, glibenclamide's ameliorative effect on body weight was statistically significant ($p < 0.05$). In contrast, there was no difference in weight between the diabetes group treated with *El:Pg* extracts and the untreated DC group ($p>0.05$).

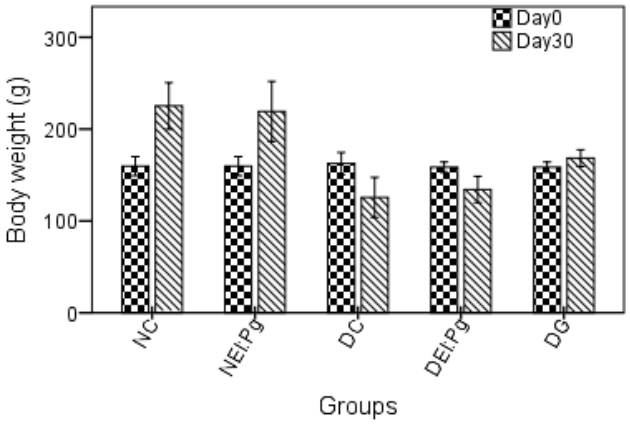

Fig. 2. Effect of *El:Pg* (2:1) on body weight of different animal groups after 30 days of administration. Values = mean ± SD, n = 5. The body weight values of all the animal groups before streptozotocin inducement were considered zero time.

The degradation of "structural proteins during DM has a significant impact on body weight which caused the significant weight loss seen in the STZ-induced diabetic rats in this study (Zafar & Naqvi, 2010; Zari & Al-Thebaiti, 2018). Another factor that contributes to body weight loss in STZ diabetic rats is gluconeogenesis, which is linked to increased muscular atrophy and protein loss from tissues (Patel *et al*., 2014). The STZ-induced diabetic rats in this study showed a substantial reduction in body weight that began in the second week. These results are consistent with earlier research, which showed" that diabetic rats left untreated had a severe loss in body weight (Kalaivanan & Pugalendi, 2011).

El:Pg (2:1) oral treatment increased the body weight of diabetic rats as seen in Figure 2. Improvements in insulin secretion and glycemic management may explain why diabetic rats gain weight following treatment (Eliza *et al*., 2009). The ability of these therapies to lower hyperglycemia was determined to be the reason why the preventative impact of glibenclamide was found to be much greater than that of *El:Pg* (2:1) plant extracts. However, numerous researchers have supported the results of this study by claiming that *E. longifolia* and P. granatum extracts have antidiabetic properties (Loizzo *et al*., 2019; Talbott, 2019; El Deeb *et al*., 2021).

Relative liver weight. The DC groups had larger liver weight relative to body weight than the normal groups as seen in Figure 3. In comparison to the NC group, the DC group had considerably higher liver weight relative to body weight ($p < 0.05$) while the difference between the diabetic groups treated with *El:Pg* (2:1) and glibenclamide and the untreated DC group was lower ($p < 0.05$).

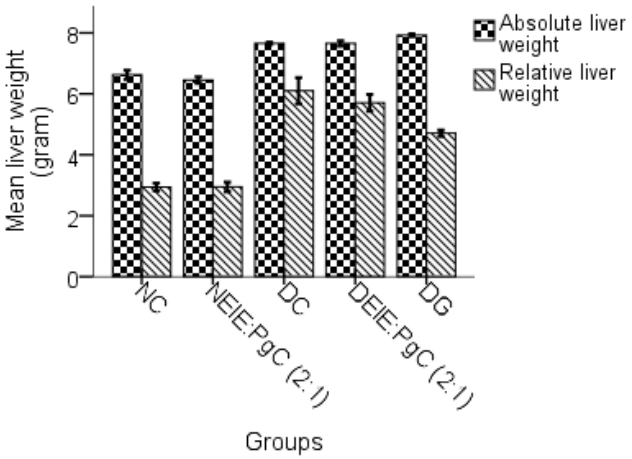

Fig. 3. Liver weight of all the animal groups (n = 5) at day 30. DC group was compared with the NC group, while the diabetic-treated groups were compared with the related values.

Despite a drop in the mean body weight, this study also showed a considerable rise in liver weight relative to body weight. The current conclusion is consistent with findings from earlier studies showing that rats with diabetes caused by STZ had an increase in relative liver weight (Zafar & Naqvi, 2010). *El:Pg* (2:1) plant extracts and glibenclamide given orally to diabetic rats restored alterations in body weight and relative liver weight as seen in Figure 3. However, the increase in liver-to-body weight could be due to hepatic hypertrophy caused by DM, which increased the level of liver triglycerides (Zafar & Naqvi, 2010).

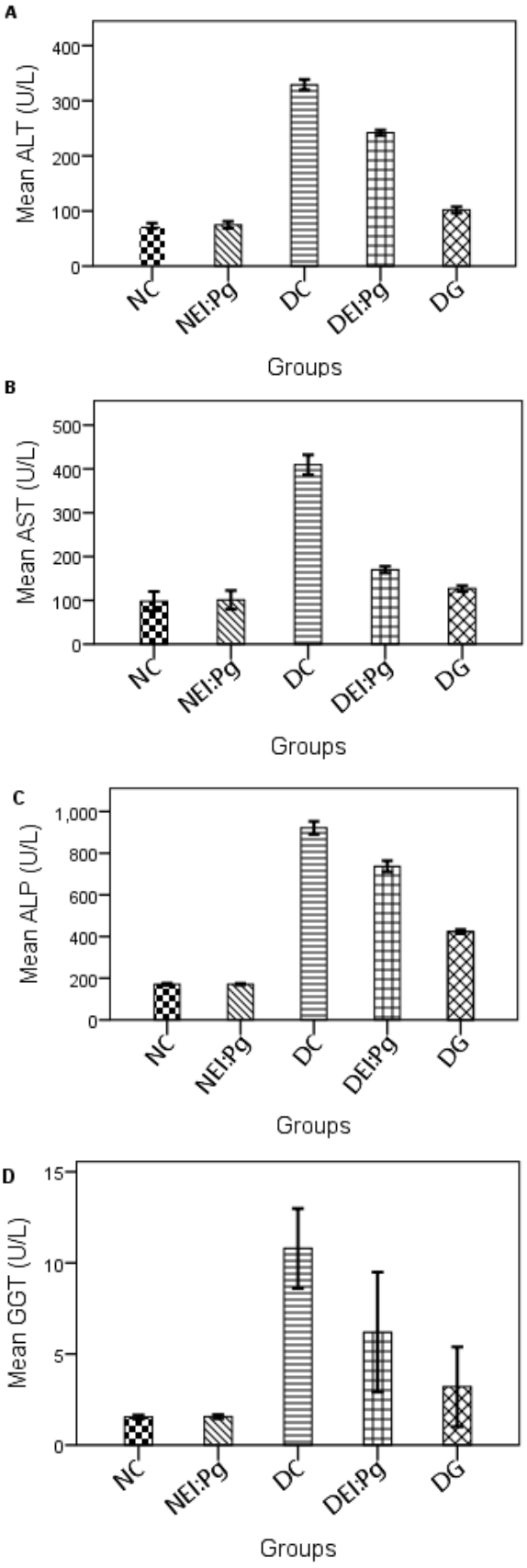

Fig. 4. Levels of plasma liver enzymes of different animal groups at day 30: (a) ALT, (b) AST, (c) ALP, and (d) GGT.

Liver function tests

Liver enzymes (ALT, AST, ALP, and GGT).The diabetic control group showed significantly higher "levels of plasma AST, ALT, ALP, and GGT concentrations compared to the normal control group ($p < 0.05$). Diabetes rats that received *El:Pg* (2:1) at the dose of 200 mg/kg BW had significantly lower plasma levels of the liver enzymes compared to the control group ($p < 0.05$). However, glibenclamide treatment significantly reduced the plasma levels of the liver enzymes compared to the DC group (Fig. 4 A-D)." El: Pg (2:1) treatment did not significantly alter the liver enzymes in non-diabetic or healthy control rats.

Protein profile. For the serum total protein, albumin, and globulin level, the observed values were considerably lower in the DC group compared to the NC group (p 0.05). Data demonstrated that the treatments with glibenclamide and *El:Pg* (2:1) extracts had a significant ameliorative impact on the treated animals compared to the non-treated diabetic groups (Fig. 5 A-C).

The diabetic rats showed high levels of liver enzyme activities following oral glibenclamide and *El:Pg* (2:1) extracts administration (Fig. 4). The level of activity of the enzymes was similarly reduced following treatment with *El:Pg* (2:1) extracts when compared to the DC group ($p < 0.05$). According to Manna *et al*. (2010), DM is frequently linked to liver damage and causes different forms of liver diseases, including abnormal liver enzyme activity, NAFLD, liver cancer, and liver cirrhosis (Tolman *et al*., 2007). Liver function tests are mostly based on the determination of the serum levels of four enzymes which are AST, ALT, ALP, and GGT as they are indicators of active hepatic diseases. For instance, transaminase levels are tremendously high during active inflammatory hepatocellular diseases. As a result, a rise in AST, ALT, ALP, and GGT plasma activity may be primarily caused by the leakage of these enzymes from the liver cytosol into the blood" stream (Eliza *et al*., 2009).

The current study also found that throughout the study, daily oral administration of *El:Pg* (2:1) extracts and glibenclamide decreased the liver enzyme activities and protein levels of diabetic rats (Figs. 4a-d and 5a-c). Hepatocyte damage alters the transport and permeability functions of the hepatocytes and causes the release of liver cell enzymes into the bloodstream (Eliza *et al*., 2009).

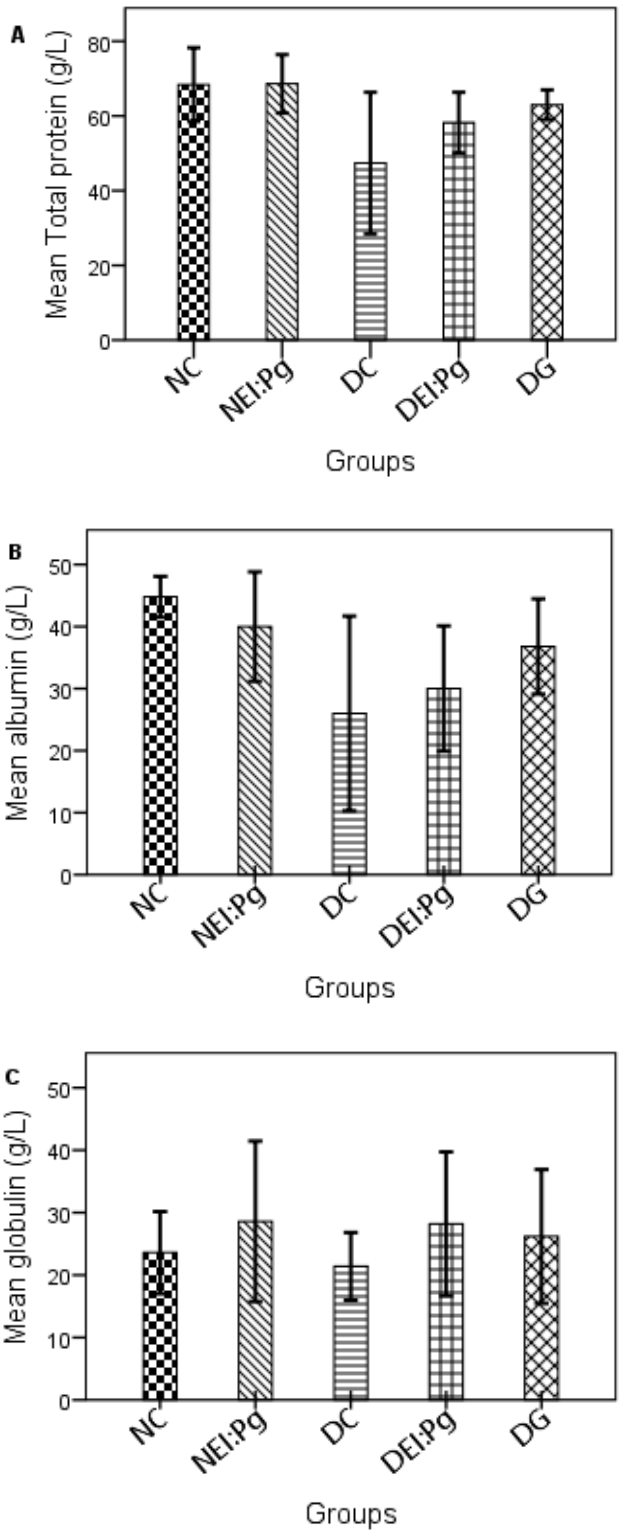

Fig. 5. Plasma protein levels of the different animal groups after the study period: (a) total protein, (b) albumin, and (c) globulin. *El:Pg* (2:1) extracts (oral administration of 200 mg/kg BW); DG: glibenclamide (0.6 mg/kg BW)-treated, diabetic rats.

Histopathological study

The liver sections of the NC and *El:Pg* (2:1) treated animals showed the normal hepatic features seen in other mammals upon staining with H&E and examined histologically. However, there was slight vacuolation in the cytoplasm of the hepatocytes of the DC group; also noted was congestion in the portal vein, sinusoid, and central vein, as well as mild hepatic cord disarray. These changes were not observed in the liver section of the diabetic rats treated with *El:Pg* (Fig. 6 a-d).

These results were in line with some histological abnormalities seen in STZ-induced diabetes in earlier research (Mahmoud & Sakr, 2013; Motshakeri *et al*., 2014). Meanwhile, glibenclamide and *El:Pg* (2:1) plant extracts-treated groups showed close to normal liver structure and pathological alterations, demonstrating that the treatments shielded the liver from DM-related damages. A protective role for *El:Pg* (2:1) plant extracts in hyperglycemia-related liver damage has been suggested by the restoration of some of these histological abnormalities following treatment with *El:Pg* (2:1) extracts.

During the final days of the experiment in the current investigation, improvements were seen in the examined parameters. To assess the therapeutic impact of long-term *El:Pg* (2:1) plant extract consumption, the experiment duration could be extended for a few more weeks. This finding supports the notion that both treatments had hepatoprotective effects on diabetic rats. The antioxidant and antihyperglycemic properties of palmitic acid, isocaproic acid, and stearic acid, which are present in *El:Pg* (2:1) plant extracts, may contribute to their hepatoprotective effects as earlier reported (Miguel *et al*., 2010; Iranshahy *et al*., 2017; Thu *et al*., 2018; Mahmood *et al*., 2019; Osama *et al*., 2020).

CONCLUSION

This research has demonstrated that daily oral administration of *El:Pg* (2:1) extracts to diabetic rats improved blood glucose levels, liver relative weight, body weight, liver biomarkers, and histological changes of the liver. As a result, more research into the *El:Pg* (2:1) extract's components is necessary to determine whether they are both safe and effective for treating diabetes mellitus and its pathological effects. It is suggested that more research be done to identify the substances that might be specifically responsible for the effects and the underlying molecular pathways.

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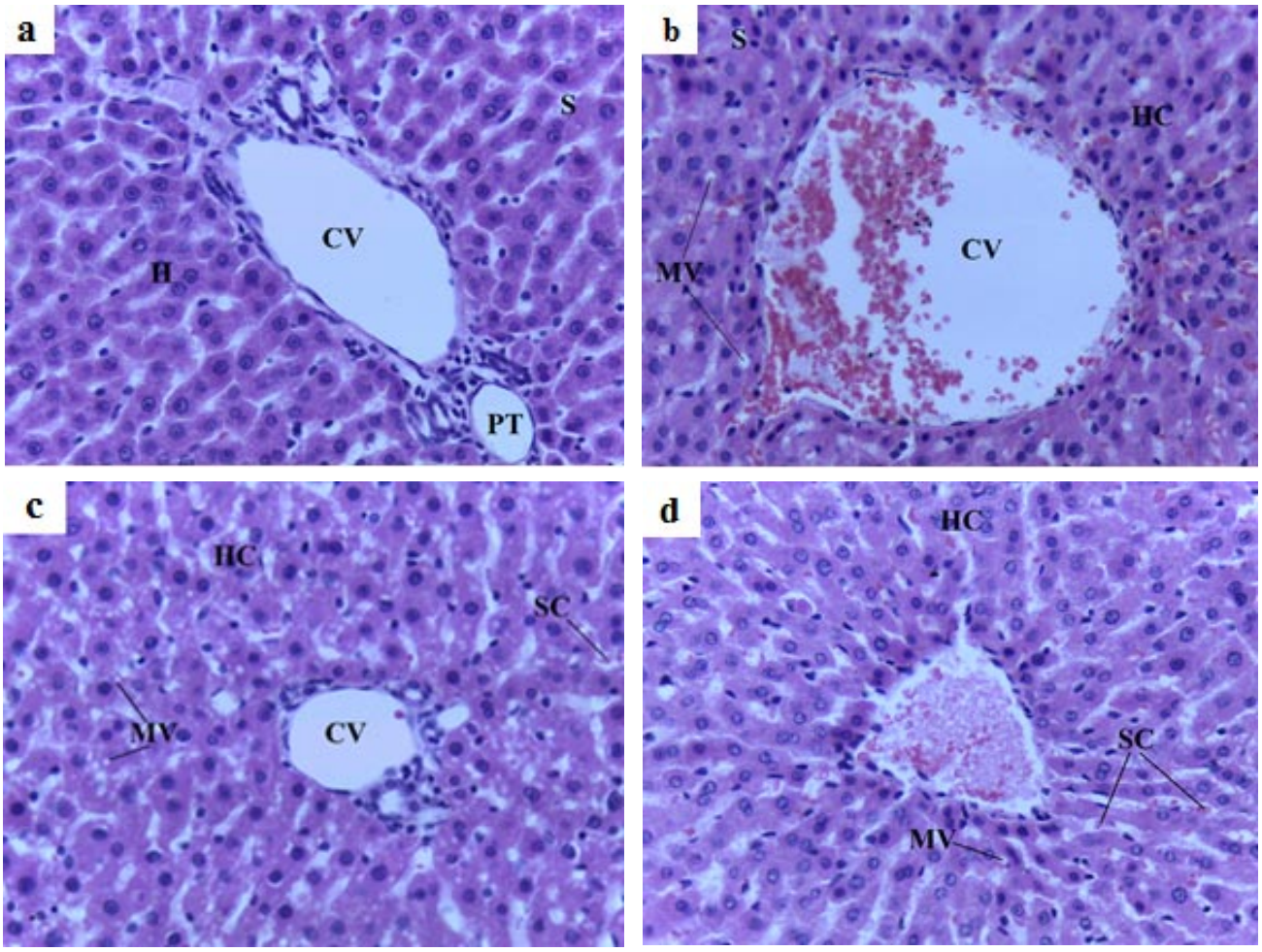

Fig. 6. Histological sections of the liver of rats from different treatment groups (a): normal control rats showing normal "liver features, with the hepatocytes (H) normally arranged in sheets, plates, and cords surrounding the central vein (CV) and portal triad (PT). The liver cells are arranged in cords that extend from the CV to the PT (separated from each other by sinusoids (S)); b): liver sections of STZ diabetic rats showing hepatocytes with mild vacuolation in the cytoplasm (MV)," congestion of CV, PV, and sinusoid (SC) (the hepatic cord also showed mild disarrangement; (c): liver sections of diabetic rats exposed to *El:Pg* (2:1) extract. There was moderate to severe cytoplasmic vacuolation of the liver cells, as well as sinusoid congestion and mild HC disarrangement. (d): liver sections of diabetic rats treated with glibenclamide; the sections showed mild cytoplasmic vacuolation in the cytoplasm of the liver cells, as well as SC and mild HC disarrangement (X400).

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RESUMEN: La diabetes mellitus (DM) se considera un síndrome metabólico de múltiples etiologías que actualmente se trata con diversas marcas de medicamentos recetados, cada uno con sus propios efectos negativos. Por ello, se están explorando alternativas naturales a base de plantas medicinales como remedios para el tratamiento de la DM debido a sus escasos o nulos efectos negativos en el organismo. En este trabajo, se estudiaron los efectos de la combinación de extracto de cáscara de cloroformo de *Punica granatum* (*Pg*) y extracto de raíz etanólica de *Eurycoma longifolia* (*El*) en una proporción combinada de 2:1 sobre la función hepática en ratas con diabetes inducida por estreptozotocina (STZ). Las ratas macho adultas Sprague Dawley se dividieron en 4 grupos (n = 5); las ratas del Grupo 1 se mantuvieron como control normal; las ratas del Grupo 2 se dejaron sin tratar tras la administración de STZ (control diabético positivo); las ratas del Grupo 3 recibieron tratamiento oral con glibenclamida para la diabetes tras la inyección de STZ; y las ratas del Grupo 4 recibieron los extractos combinados (200 mg/kg de *El:Pg* en una proporción 2:1) durante 25 días consecutivos. Se midieron

los niveles de glucosa en sangre y los pesos del cuerpo y del hígado de las ratas de cada grupo al final del período de estudio. Se realizó un análisis histológico del hígado y una investigación de la función hepática. En ratas diabéticas, los extractos combinados *El:Pg* (2:1) aumentaron la tasa de pérdida de peso y los niveles de glucosa en sangre en ayunas (p 0,05); las ratas diabéticas también tuvieron niveles reducidos de enzimas hepáticas (AST, ALP, ALT y GGT) en comparación con las ratas control diabéticas (p < 0,05). El estudio histológico mostró congestión sinusoidal, vacuolización de severa a moderada en el citoplasma de las células hepáticas y una leve alteración del cordón hepático en las ratas tratadas con el extracto combinado de *El:Pg*. Este estudio demostró experimentalmente las propiedades antihiperglucémicas del *El:Pg* (2:1), que podrían ser útiles para detener la progresión de la diabetes mediante acciones hepatoprotectoras.

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

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