



Original article

Ellagic acid regulates hyperglycemic state through modulation of pancreatic IL-6 and TNF- α immunexpressionManal M. Elbandrawy^a, Osama Sweef^{b,e}, Doaa Elgamal^a, Tarek M. Mohamed^a, EhabTousson^b, Rehab M. Elgharabawy^{c,d,*}^a Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt^b Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt^c Department of Pharmacology & Toxicology, Faculty of Pharmacy, Tanta University, Tanta, Egypt^d Department of Pharmacology & Toxicology, College of Pharmacy, Qassim, Qassim University, Saudi Arabia^e Department of Medicine, MetroHealth Medical Center, School of Medicine, Case Western Reserve University, Cleveland, Ohio-44109, United State

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ABSTRACT

Background: Type 2 diabetes (T2DM) is a chronic metabolic disorder. Although therapeutic pharmaceutical agents continue to advance, herbal medicines are potential complementary treatments for the promotion of glucose homeostasis, with minimal adverse effects. Conventionally, ellagic acid (EA) has been utilized for the therapy of a range of pathologies owing to its anti-inflammatory and anti-diabetic actions.

Objective: The aim of this study is to determine the activity of EA on serum α -amylase and lipase titers, and on pancreatic tumor necrosis factor- α (TNF- α), proliferating cell nuclear antigen (PCNA) and interleukin-6 (IL-6) concentrations using the streptozocin-induced T2DM rodent model.

Methods: EA extract synthesized from fresh strawberry fruit was employed for therapy. 50 adults male Wistar rats were randomized into either control, EA, diabetic, co-treated or post- treated cohorts.

Results: EA diminished fasting blood glucose levels, altered lipase, amylase, IL-6, PCNA and TNF- α expression and enhanced islet cell renewal, insulin, and immunoreactivities.

Conclusion: Inflammatory indicators are elevated in the presence of T2DM. Extract of EA has overall tissue reparative and safeguarding properties, as indicated by the augmented β - cell population and enhanced glucose homeostasis. Thus, EA may be an innovative treatment approach for the maintenance of normoglycemia in individuals with T2DM.

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1. Introduction

An endocrine gland, the pancreas contributes to the maintenance of normoglycemia. It is comprised of two principal types of tissue: (i) exocrine tissue, which liberates enzymes synthesized within the pancreas in order to extract important nutrients and to aid the gastrointestinal breakdown of lipids,

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carbohydrates and proteins; and (ii) endocrine tissue, which is comprised of cells which manufacture glucagon (α -cells, 15–20%), somatostatin (δ - cells, 3–10%), insulin (β -cells, 65–80%), pancreatic polypeptide (PP-cells, 1%) and ghrelin (ϵ - cells) (Arutyunyan et al., 2020).

Diabetes mellitus (DM) is a condition that occurs following impairment of the β -cells of the pancreas, leading to diminished insulin release and abnormal function of insulin receptors; it may impact one or more of these physiological functions (El Barky et al., 2018). Any compromise in pancreatic activity can lead to the evolution of either type 1 (T1DM) or type 2 (T2DM) DM (Arutyunyan et al., 2020).

T2DM is a well-recognized enduring disorder that encompasses a cohort of metabolic conditions of which the hallmark is resistance to insulin (Arnold et al., 2018). Globally, in the region of 450 million individuals are impacted by T2DM. It is anticipated that 7000 million people may be affected by 2045 if the recent rise

in prevalence persists (Saeedi et al., 2019). The latter has increased by two-fold over the last 60 years, generating notable health concerns including both disability and death (Gao et al., 2020). The onset of T2DM is associated with contemporary lifestyle choices, such as lack of physical exercise, poor diet and an elevated body mass index, together with inherited genetic traits. If this condition remains untreated, a range of pathologies and chronic complications ensue, ultimately leading to the patient's demise (Lotfy et al., 2016).

Persistently elevated serum glucose levels are associated with both microvascular and macrovascular pathologies. The former may affect the kidneys, nervous system, e.g., diabetic foot, and retina, whereas the latter encompass cerebrovascular, cardiovascular and peripheral vascular diseases, respectively. Hyperglycemia is additionally related to a poor life quality and mortality (Li et al., 2020). The principal complications that cause fatalities in T2DM are those affecting the microvasculature; these have been connected to blood oxidative stress and poor control of serum glucose titers. Oxidative stress arises owing to the triggering of superoxide liberation in response to alterations in the mitochondrial electron transport chain and amplified NADPH oxidase activity. Additionally, hypertension is viewed as one of the most prevalent T2DM complications, occurring in 70.9% of patients. Hepatic pathologies, e.g., steatosis and fibrosis, are also correlated with the presence of T2DM (Zhao et al., 2018). Inflammation is a consequence of hyperglycemia; it can be recognized by the elevation of a number of inflammatory indicators, e.g., high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and interleukin-18 (IL-18). Inflammatory activity can underlie insulin resistance, which compounds DM; it also adversely influences pancreatic β -cell activity (Mardiha et al., 2015).

IL-6, a cytokine with numerous activities, has been connected to the pathogenesis of T2DM. Increased serum IL-6 titers are an autonomous marker for T2DM; it has been postulated that these contribute to inflammatory processes, impairment of β -cells and peripheral resistance to insulin. IL-6 additionally seems to have anti-inflammatory properties; an increasing number of studies have suggested that it promotes glucose homeostasis (Akbari and Hassan-Zadeh, 2018).

TNF- α is a cellular signaling protein that also participates in the inflammatory process and a major cytokine in the acute phase reaction. It is a key player in T2DM and insulin resistance, inhibiting insulin-induced tyrosine phosphorylation on the insulin receptor substrate-1 β -chain and insulin receptor. Both TNF- α and IL-6 are major actors in the evolution of insulin resistance and the underlying disease processes (Ramadan et al., 2017).

A broad spectrum of useful chemical substances for the prophylaxis and therapy of numerous pathologies can be extracted from medicinal herbs (Alotaibi et al., 2021). Such natural compounds have been utilized in conditions, such as DM, hypertension, malignancy and cardiac pathologies (Abd Eldaim et al., 2021; Aldubayan et al., 2019). Currently, in excess of 800 species have been the subject of investigation; their glucose-lowering properties have been documented (El-Gharbawy et al., 2016).

Strawberries, or *Fragaria* \times *ananassa*, contain significant amounts of ellagic acid (EA), i.e., 40 mg/100 g. This fruit is considered to be one of the most significant EA sources. EA is a compound which has been reported to have multiple advantages in terms of well-being. A naturally arising polyphenol, with the chemical formula C₁₄H₆O₈,

EA is attributed with anti-DM, anti-inflammatory and neuroprophylactic characteristics (Farbood et al., 2019).

The aim of this research is to investigate the prophylactic and clinical treatment potentials of EA with respect to pancreatic disorders.

2. Materials and methods

2.1. Extraction of ellagic acid from strawberry fruit

Ten fresh strawberries, minus the seeds, were liquidized in a food processor. Single 0.5 g aliquots were decanted into constrictive glass tubes with 5 ml 1 M sulphuric acid. A dry ice/2-propanol bath was utilized to freeze the samples. They were then vacuum-packed, sealed and warmed to 100 °C for the requisite period. Following hydrolysis, the tubes were returned to ambient temperature, then opened and placed in an ice bath for 10 min in order to lower the temperature further. The specimens underwent centrifugation and the supernatants were decanted. The specimen residues were cleansed two-fold with 5 ml ice-cold wash solvent, i.e., acetone/H₂O/concentrated hydrochloric acid 70:30:1 v/v/v, desiccated using nitrogen and finally, made up to a solution with 10 ml dimethyl sulphoxide. The ultimate supernatant was used for the investigation of EA (Williner et al., 2003).

2.2. Plant material

2.2.1. Determination of total phenolic and flavonoid content of extract

The Folin-Ciocalteu technique was utilized in order to identify the phenolic substances within the extract (Cheok et al., 2012). The sum of the flavonoid matter was established by employing an aluminum chloride colorimetric assay using quercetin as a reference. The results were given in mg, i.e., as mg quercetin equivalent per gram of extract (Zhishen et al., 1999).

2.2.2. Determination of 1,1-diphenyl-2-picrylhydrazyl scavenging activity and total antioxidant capacity of extract

The appraisal of radical scavenging was conducted by a well-recognized method described by Brand-Williams et al. (Brand-Williams et al., 1995), using 1,1-diphenyl-2-picrylhydrazyl free radical solution in order to assess the antioxidant potential using spectrophotometry. A phosphomolybdenum assay was employed in order to gauge the total antioxidant capacity of the extract (Prieto et al., 1999).

2.2.3. Identification of ellagic acid extracted from strawberry fruit using Fourier-transform infrared spectroscopy

This part of the methods was performed in the Microanalytical Unit, Faculty of Science, Tanta University, Egypt. Fourier-transform infrared (FT-IR) spectroscopy (JASCO FT-IR 4100 LE, Japan; range: 4000–400 cm⁻¹) was deployed in order to recognize functional moieties of the active substances. Between 1 and 2 mg of EA extract was pulverized to a powder with a mortar and then admixed with 3–4 mg potassium bromide.

2.3. Experimental animals

Fifty male adult Wistar rats of bodyweight 230 \pm 20 g were purchased from the Egyptian stock holding company for biological products of vaccines, sera and drugs (VACSERA, Helwan, Egypt). The rodents were allowed to adjust to their changed environment for the week before the study commenced. They were maintained with routine parameters, i.e., a temperature of 26 \pm 2 °C, 12-hourly diurnal cycle and free access to food and water. All experiments were performed following study procedures sanctioned by Tanta University Ethics Committee (REC) for Animal Subject Research (NHTMRI), 2020 (serial number: IACUC/ERC).

2.4. Induction of type 2 diabetes mellitus

T2DM was provoked by an intraperitoneal (ip) injection of 55 mg/kg fresh streptozotocin (STZ) (Sigma-Aldrich Corp, St. Louis, MO, USA) placed in an ice-cold 0.1 M citrate buffer with a pH of 4.5. 72 h after the STZ was administered, the onset of T2DM was verified by assaying fasting serum blood glucose (FBG) levels from tail vein samples, utilizing a blood glucose meter (Accu-Check Performa, Roche Diagnostic, Germany). Rodents were categorized as having T2DM if FBG > 200 mg/dL.

2.5. Experimental planning

The fifty rats were allocated to five cohorts that received the following interventions:

- (i) Group I: negative controls, which underwent no intervention for 4 weeks
- (ii) Group II: EA, which received 50 mg/kg/day oral EA for 30 days (Corbett et al., 2010).
- (iii) Group III: diabetic, which were administered a single ip injection of STZ sodium citrate solution (Furman, 2015).
- (iv) Group IV: cotreated, which received 50 mg/kg/day oral EA for 15 days, followed by a single ip injection of STZ sodium citrate solution, and then a further 50 mg/kg/day oral EA for 15 days
- (v) Group V: post-treated, which had a single ip injection of STZ sodium citrate solution and then 50 mg/kg/day oral EA for 30 days

Following the conclusion of the experiment, the rats were starved overnight, anaesthetized with (ip) sodium pentobarbital and then euthanized. Blood samples were obtained from the retroorbital venous plexus and collected in plain glass tubes to estimate the biochemical parameters. Blood serum was separated by centrifugation at 3000 rpm for 15 min. Tissue specimens from the pancreas were extracted with care and split into segments for histology and immunohistochemistry analyses.

2.6. Biochemical examination

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental studies (Tveden-Nyborg et al., 2021). A spectrum diagnostics kit (Obour City, Egypt) was employed in order to acquire an enzymatic measure of FBG. Techniques described by Kumar and Gill (Kumar and Gill, 2018) were utilized so as to identify α -amylase and lipase concentrations, respectively.

2.7. Histopathological examination

A 24-hour immersion in 10% neutral buffer formalin was used to fix the pancreatic tissue prior to its embedment in paraffin wax, according to the technique reported by Dury and Wallington. The specimens were then sectioned into 5 μ m slices; haematoxylin and eosin staining were performed (Tousson, 2016). The sections were viewed using light microscopy (Leica research microscope; Leica EC3 camera, Leitz Wetzlar, Germany) in order to assess the configuration of the pancreatic islets. Digital photomicrographs were acquired at a range of magnifications.

Table 1

Phytochemical profile of the methanolic extract of strawberry fruit.

Total polyphenol content	642.6 mg gallic acid equivalent per 100 g sample
Total flavonoid content	98.5 mg quercetin equivalent per 100 g sample
Antioxidant activity	59.81%
DPPH radical scavenging activity IC50 (dry sample)	7.5

2.8. Immunohistochemical detection of proliferating cell nuclear antigen, insulin, tumor necrosis factor- α and interleukin-6 immunoreactivities

The following immunohistochemical assays were performed, using an avidin biotin complex (ABC) technique in all cases (Elite-ABC, Vector Laboratories, CA, USA) in order to identify antibody staining for the following: (i) proliferating cell nuclear antigen (PCNA) (antibody dilution 1:100; DAKO, Japan Co., Tokyo, Japan) Tousson et al. (Tousson et al., 2011); (ii) insulin (antibody dilution 1:100; Monoclonal Anti-insulin, No. I2018, Sigma) Suarsana et al. (Suarsana et al., 2016); (iii) TNF- α (antibody dilution 1:200; DAKO Japan Co, Tokyo, Japan) El-Masry et al. (El-Masry et al., 2020a) and (iv) IL-6 (antibody dilution 1:200; rabbit anti-IL-6 serum, Abcam, product Ab6672) Rajčáni et al. (Rajčáni et al., 2019).

2.9. Statistical evaluation

The analysis of results was done using the Statistical Package for the Social Sciences (SPSS software version 16). Data were presented as the mean \pm standard error of mean (SEM) and statistically analyzed by one-way ANOVA (Analysis of Variance) followed by Dunnett test. Dunnett test comparisons were performed to assess the significance of differences between groups. Unpaired T-test was performed to compare the significant difference between groups. The criterion for statistical significance was set at $p < 0.05$.

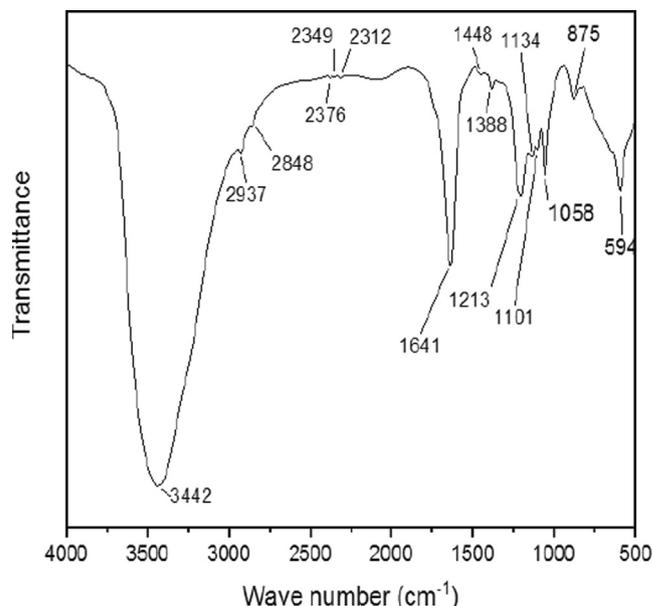


Fig. 1. FTIR spectra of the ellagic acid.

Table 2
Impact of treatment with ellagic acid extract on serum FBG, α -amylase and lipase titres in the differing rodent cohorts.

	Control	Ellagic	Diabetic	Co-treated	Post-treated
Lipase (IU/L)	46.0 [#] ±1.2	45.3 [#] ±0.9	26.25*±0.5	43.33 [#] ±0.8	46.33 [#] ±1.9
α -amylase (IU/L)	647.5 [#] ±2.0	632.5 [#] ±1.3	815.7*±3.2	621.7 [#] ±1.5	524.5 [#] ±2.5
FBG (mg/dl)	89.7 [#] ±0.6	88.5 [#] ±0.8	292.1*±5.4	287.3*±3.5	182.3 [#] ±10.1

Data are expressed as mean ± SEM of 10 observations. Significant difference from the control group at *p < 0.05. Significant difference from the diabetic group at [#]p < 0.05.

3. Results

3.1. Phytochemical profile of strawberry fruit

The phytochemical profile of the methanolic strawberry extract is indicated in Table 1, which lists the total phenolic and flavonoid substances present, the total antioxidant activity and the IC50.

3.2. Characterization of extracted ellagic acid

The EA, extracted from the strawberry fruit, was analyzed using FT-IR (Fig. 1). The aromatic C–H out-plane bending was demonstrated by the absorption zeniths seen at 874 cm⁻¹ and 594 cm⁻¹. C–C–O and C=O stretching were indicated by the zeniths at 1213 cm⁻¹ and 1448 cm⁻¹, respectively, the latter indi-

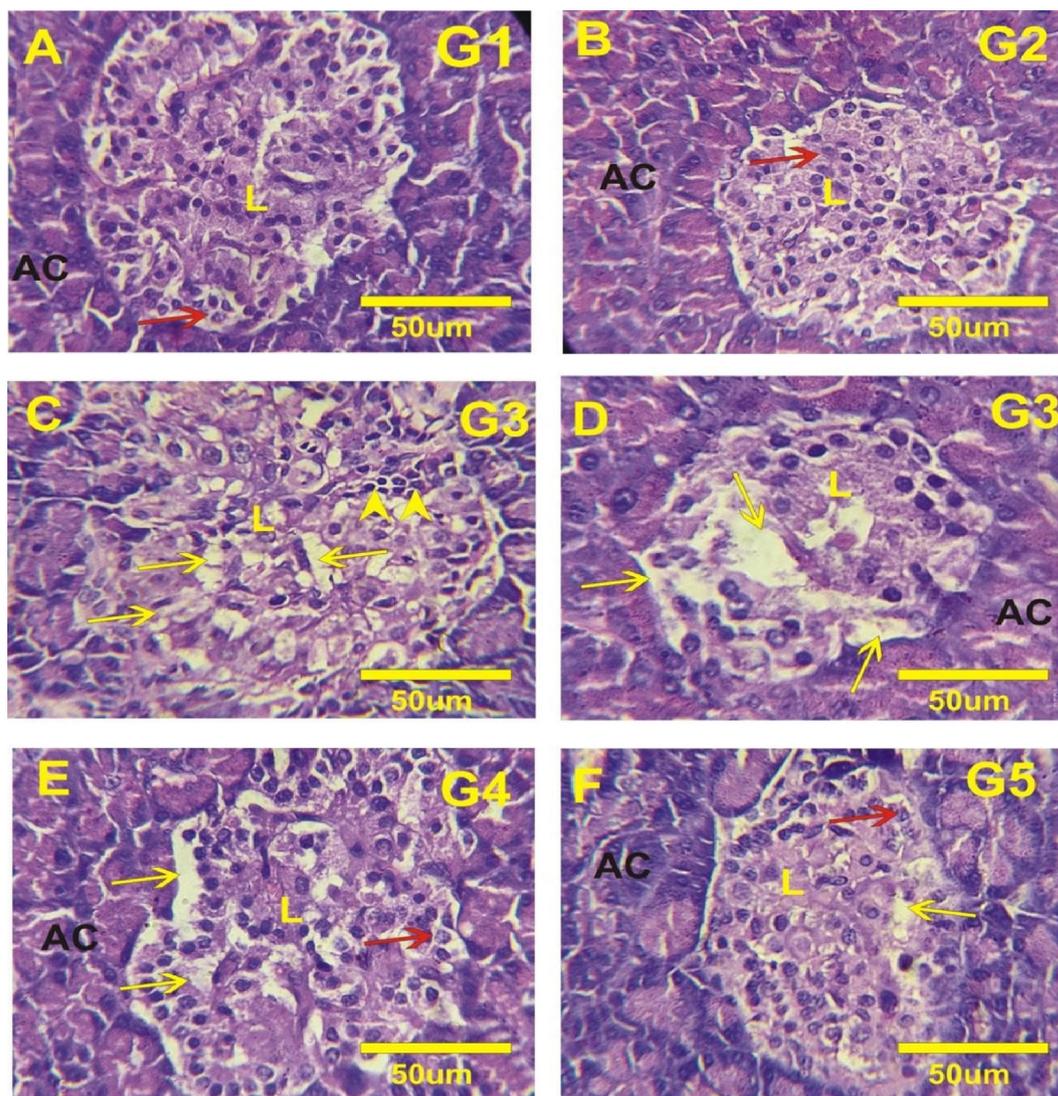


Fig. 2. Photomicrographs of rat's pancreas sections in the different experimental groups stained with H&E. A&B: Normal structure of the pancreas consisted of exocrine (Acinar cells; AC) and endocrine (islets of Langerhans; L) portions in control and EA groups. C&D: Diabetic rats revealed decreased islets size, decreased β -cells number and degenerated entering connective tissue (arrows) sheet with irregular islets shape. E: Pancreatic sections in co-treated. F: Post treated revealed normal shape and increased β -cells number.

cating unsaturated lactone. C=C–C vibrations were evident with a hump at 1641 cm^{-1} . A peak at 1383 cm^{-1} reflected a distortion band external to the O–H plane. Asymmetric and symmetric C–H stretching implying $-\text{CH}_3$ were represented by peaks at 2937 cm^{-1} and 2848 cm^{-1} , respectively, whereas the broad zenith noted at 3442 cm^{-1} could be attributed to the stretching vibrations of the OH moiety.

3.3. Impact of ellagic acid on metabolic activity

Table 2 revealed that; elevation ($p < 0.05$) in FBG and α -amylase concentrations in diabetic rats in contrast to the control group, however, serum lipase titers were diminished in the latter cohort. In the co-treated and post-treated groups, EA reduced both FBG and α -amylase levels whereas lipase concentrations were enhanced when judged against group III.

3.4. Potential effect of ellagic acid on pancreatic tissues

The pancreatic architecture appeared normal in the control rats and in the cohort treated with EA; pancreatic acinar cells containing amphophilic cytoplasm and basal nuclei were demonstrated. A plethora of β -cells, distinguished by their spherical appearance and globular nuclei were present within the islets of Langerhans, which were also of normal morphology, evidenced by a typical circular appearance caused by the connective tissue elements. In the rats with STZ-induced DM, the islets were reduced, contained a smaller population of β -cells and degenerated entering connective tissue, the connective tissue planes were degraded creating a lack of islet uniformity. The co-treated and post-treated murine cohorts exhibited a plethora of islets of a practically normal morphology, together with cellular renewal. Enhancement of several of the islets of Langerhans were noted in the post-treated group; essentially normal nuclei were noted (Fig. 2 A-F).

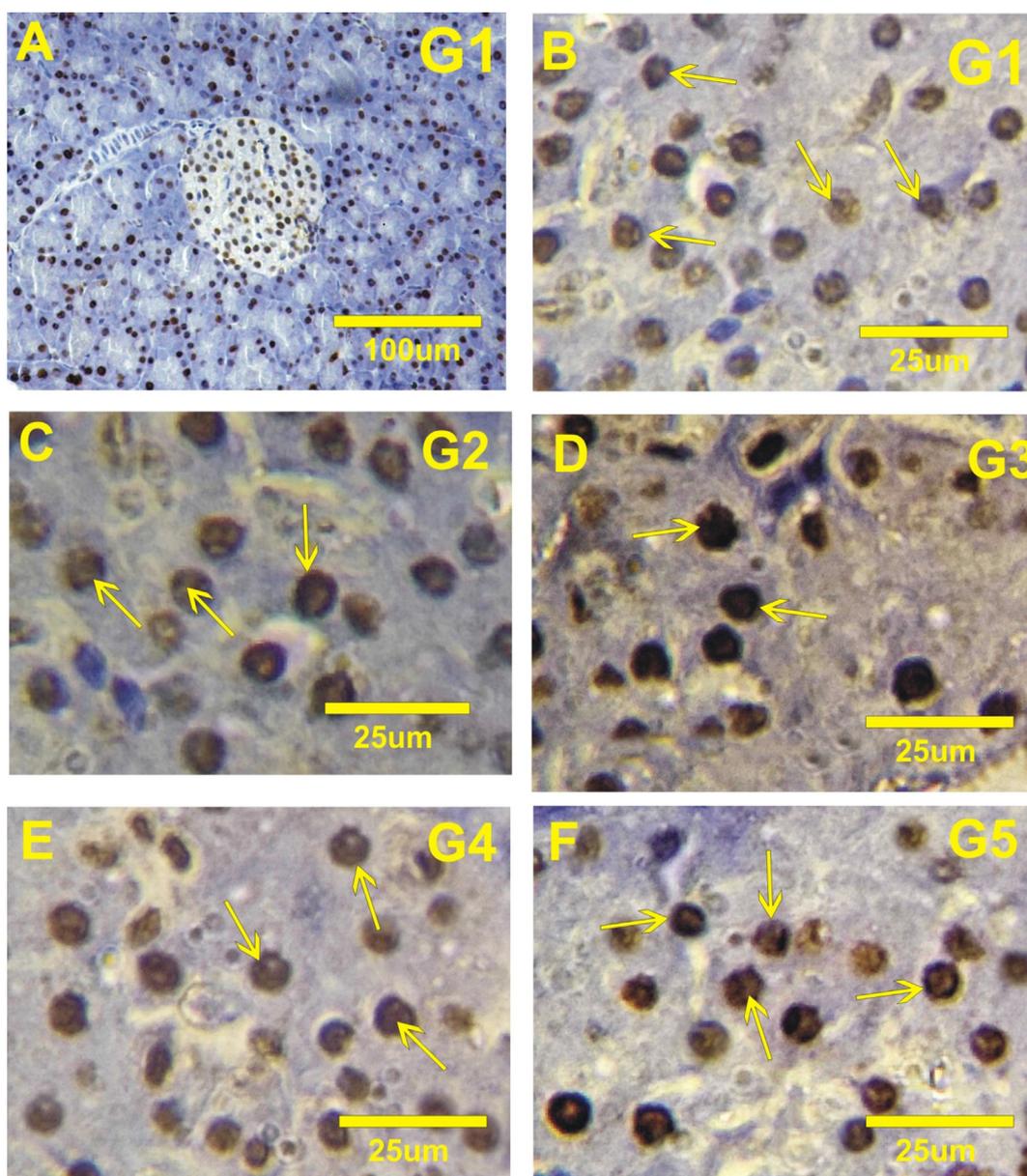


Fig. 3. Photomicrographs of rats pancreas sections in the different experimental groups stained with PCNA. A-C: Strong positive reaction for PCNA immunoreactivity in control and ellagic acid groups. D: Mild to moderate PCNA positive reactions in diabetic group. E: Moderate PCNA positive reactions in co-treated group. F: Strong PCNA positive reactions in post-treated group.

3.5. Impact of ellagic acid on proliferating cell nuclear antigen, insulin, tumour necrosis factor- α and interleukin-6 immunoreactivities

A robust positive reaction for immunoreactivity associated with PCNA was seen in the pancreatic specimens from the control group and the cohort which received EA (Fig. 3A, 3B). In the diabetic group, mild-moderate positive reactions for PCNA were noted (Fig. 3C, 3D). Moderate and robust reactions for PCNA were demonstrated in the co-treated and post-treated cohorts, respectively. (Fig. 3E, 3F).

Alterations in insulin immunoreactivity seen in the β -cells are illustrated in Fig. 4. Strong positive reactions were noted in the control and EA cohorts (Fig. 4A, 4B), mild-moderate staining was observed in the diabetic rats (Fig. 4C, 4D) whereas those that were in the co-treated and post-treated intervention arms demonstrated moderate to strong staining for insulin (Fig. 4E, 4F).

The immunoreactivity relating to TNF- α in the islets of Langerhans for the varying cohorts is depicted in Fig. 5. Antibody staining for TNF- α was either absent or only marginally present in the control rats and those receiving EA (Fig. 5A, 5B). A moderately positive reaction was seen in the murine with induced diabetes (Fig. 5C). The rodents in the co-treated and post-treated cohorts evidenced a moderate to robust positive reaction for insulin (Fig. 5D, 5E).

The immunoreactive results for IL-6 within the pancreatic β -cells are shown in Fig. 6. Positive reactions of a mild to moderate nature were evident in the control and EA cohorts (Fig. 6A, 6B), whereas strong staining was present in pancreatic sections from the diabetic rats (Fig. 6C, 6D). Co-treated and post-treated murine exhibited moderate-strong and moderate staining results for IL-6, respectively (Fig. 6E, 6F).

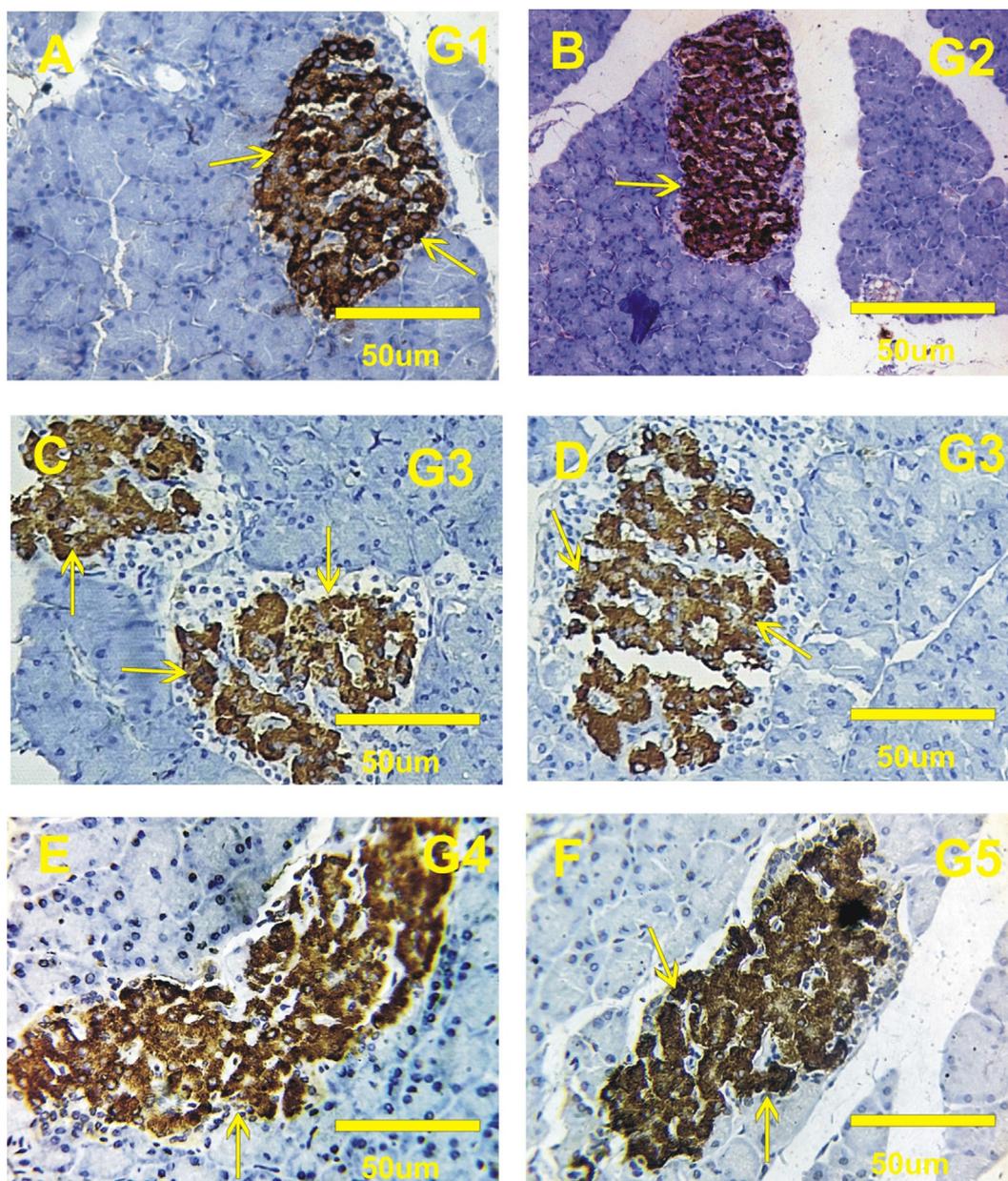


Fig. 4. Photomicrographs of rat's pancreas (β -cell) sections in the different experimental groups stained with insulin immunoreaction. A&B: Strong positive reaction for insulin immunoreactivity in control and ellagic acid groups. C&D: Mild to moderate insulin positive reactions in diabetic group. E&F: Moderate to strong positive reactions for insulin in co-treated and post-treated groups.

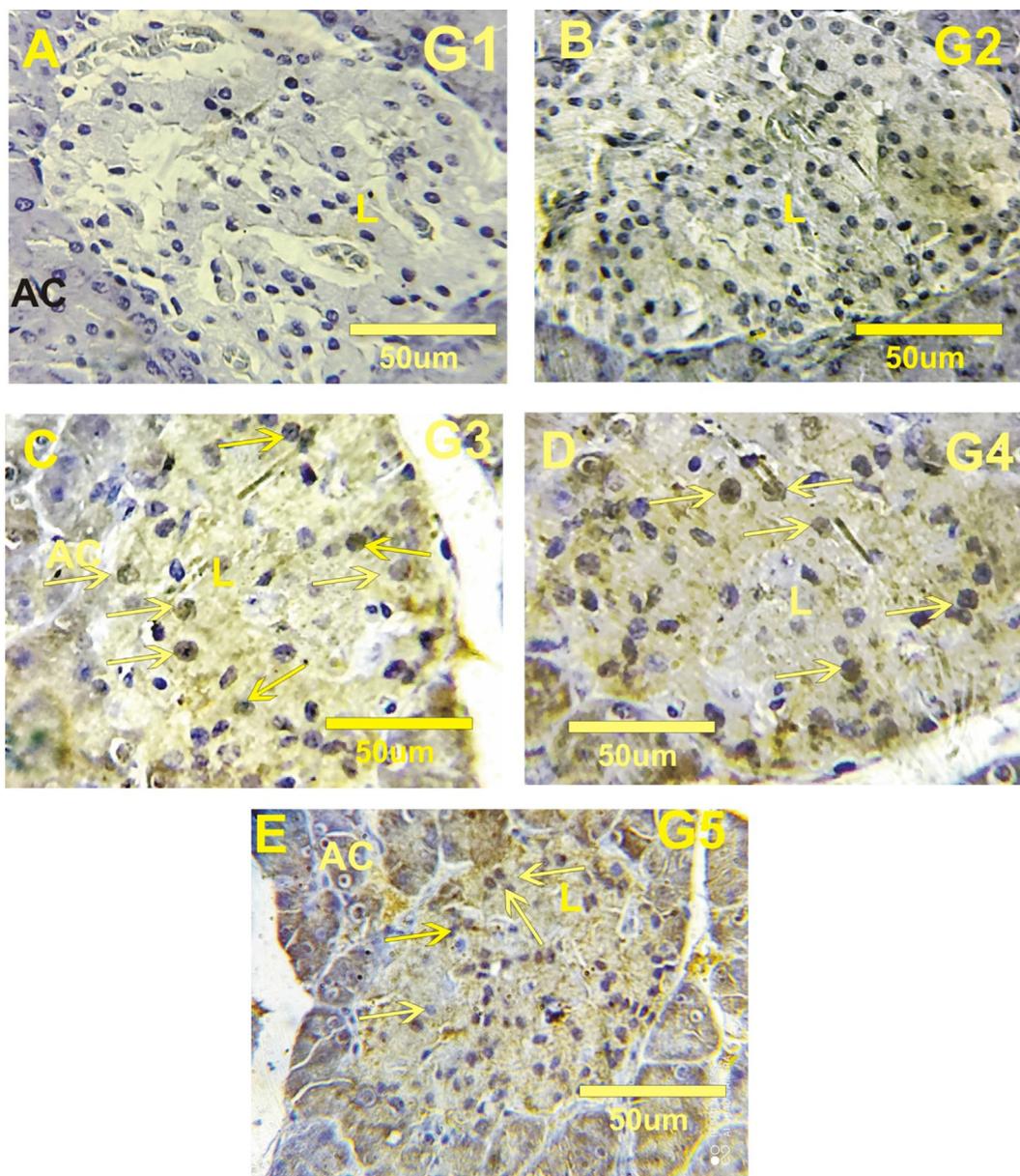


Fig. 5. Photomicrographs of rats pancreas (β -cell) sections in the different experimental groups stained with pro-inflammatory marker TNF- α immunoreactivity. A&B: Negative or faint positive reaction for TNF- α immunoreactivity in control and ellagic acid groups. C: Moderate positive reaction for TNF- α immunoreactivity in diabetic group. D&E: Moderate positive reaction for TNF- α immunoreactivity in co-treated and post-treated groups.

4. Discussion

The pathological manifestations of the enduring raised glucose titers characteristic of DM genuinely give rise to morbidity and mortality. The majority of viscera are adversely impacted by T2DM, causing functional impairment and permanent injury (El Barky et al., 2018). STZ has a targeted toxic effect on the β -cells of the pancreas, which is ascribed to the glucose residue within their chemical configuration which facilitates its entry into the cells via the low plasma membrane glucose transporter 2 receptor affinity. There are 3 main mechanisms underlying the ability of STZ to injure the β -cells: (i) decreased NAD⁺ levels as a result of carbonium ion production arising from methylation of DNA; (ii) STZ behaves as a donor of nitric acid; this is synthesized through alkylation of the DNA and subsequent injury; and (iii) free radical or reactive oxygen species (ROS) manufacture (Rajesh and Sreekala, 2020).

A phenolic substance, EA is found in various fruits, e.g., raspberries, strawberries, blueberries and walnuts. The compound has been shown to have anti-diabetic activity (Harakeh et al., 2020). In this study, EA was derived from strawberry fruit and analyzed using FT-IR spectroscopy. The obtained data were comparable to those formerly published (Mady and Shaker, 2017). The use of STZ in this research generated a murine model of T2DM, evidenced by the raised FBG in the treated animals and in keeping with earlier studies (Konsue et al., 2017). The lower FBG identified in the rodents which received the EA extract compared to the controls supported the data published by (Yeh et al., (9) (1123) (2017)), and affirmed that EA behaves as an anti- hyperglycemic agent.

Compared to controls, serum α -amylase titers were higher in the rats with T2DM, an observation aligned with previous studies. However, some workers have reported a fall in serum α -amylase levels in T2DM (Ata et al., 2015). The opposite pattern for serum lipase concentrations was noted; these were decreased in rodents

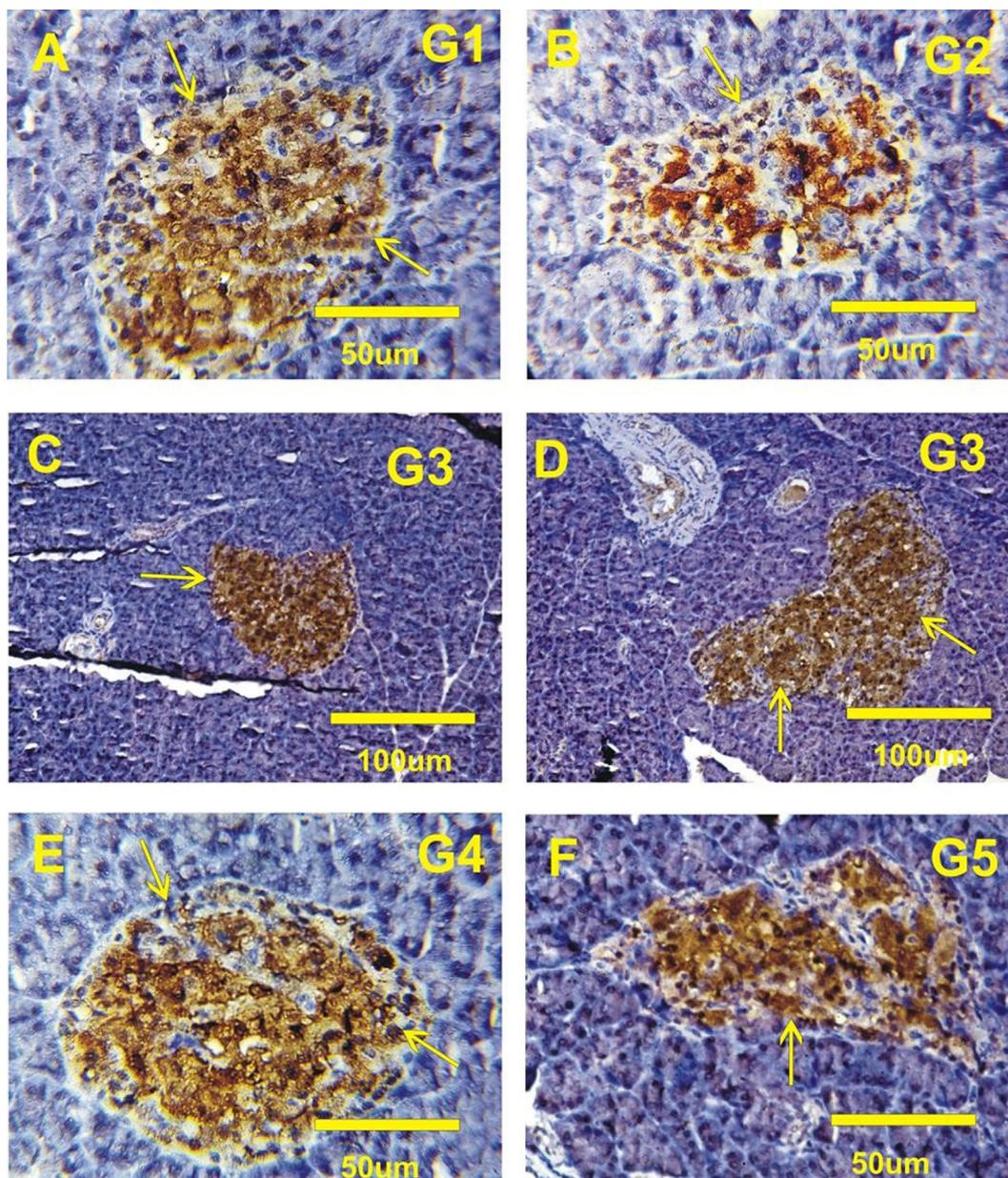


Fig. 6. Photomicrographs of rats pancreas (β -cell) sections in the different experimental groups stained with IL-6 immunorexpression. A&B: Mild to moderate positive reaction for IL-6 immunoreactivity in control and ellagic acid group. C&D: strong positive reaction for IL-6 immunoreactivity in diabetic group. E: Moderate positive reaction for IL-6 immunoreactivity in co-treated group. F: Moderate positive reaction for IL-6 immunoreactivity in post-treated group.

with T2DM when judged against controls. Previous studies have lacked consistency relating to this finding, with both lower lipase levels and raised lipase titers being reported in T2DM (Tanvi et al., 2017). Augmented exocrine acinar cell attrition and anti-insulin hormonal activity may occur in T2DM as a result of peripheral resistance to insulin. This can lead to a decrease in pancreatic exocrine enzyme manufacture and liberation. Additionally, with the onset of T2DM, the link between the exocrine acinar cells and the endocrine islets is broken; fibrosis occurs in the former and there is a less sensitive reaction to hormonal signals (Tanvi et al., 2017; Elkotby et al., 2018).

In the present work, the histopathology results from the rodents receiving EA demonstrated a number of positive changes when judged against the pancreatic sections from the rats with induced T2DM. In the samples from the latter group, the islets of Langerhans exhibited increasing numbers of large vacuoles and loss of

structural integrity, together with an attrition in β -cell population size and configuration. These morphological appearances have previously been noted by El-Shitany et al. (2014). In groups IV and V, which underwent intervention, signs of tissue reparation were evident in the β -cells, with less fibroseptal connective tissue strands, more structured and well-defined morphology as well as minimal vacuolization within the islets of Langerhans. In contrast to the rodents with induced T2DM, hemorrhagic regions were lower in number.

EA is the dilactone of hexahydroxydiphenic acid and a natural phenol antioxidant found in fruits and vegetables. EA has potential anti-inflammatory and antioxidant properties. EA is the dilactone of hexahydroxydiphenic acid and a natural phenol antioxidant found in fruits and vegetables. EA has potential anti-inflammatory and antioxidant properties. El-Shitany (Tousson et al., 2011) reported that EA has a very powerful anti-

inflammatory effect through inhibition the expression of NO, MDA, IL-1 β , TNF- α , COX-2 and NF- κ B and stimulation the synthesis of GSH and IL-10.

PCNA is an indicator of replication and functions as an adjunct to DNA polymerase δ (Oyouni et al., 2018; Ali, 2020). In this study, compared with the findings in the control animals, the diminished anti-PCNA staining and lower frequency of PCNA positive nuclei seen in the rodents with T2DM reflected diminished pancreatic islet cell replication. These results supported those of Ali (Abdul-Ghani et al., 2019). In cohorts IV and V, administered EA, the heightened PCNA staining and elevated proportion of nuclei positive for PCNA compared favorably with the findings in group III, and represented amplified cellular replication within the pancreatic islets, acini and vasculature.

A major factor in T2DM, the development of insulin resistance exacerbates raised serum glucose levels (Iliya et al., 2016). Shehan et al. (2019) described the uniform presence of insulin within islet cells in subjects with DM; the observations from group III in the current study concur. In contrast, the rodents administered EA demonstrated a rise in sensitivity to EA, as also noted by Alexandraki et al. (1084). Ongoing systemic inflammatory processes, characterized by the existence of poorly controlled serum inflammatory biomarkers, are typical of T2DM. IL-6 leads to the accrual of macrophages within fat tissue which is a frequent origin of adipocytes and macrophages. Additionally, the preponderance of β -cells undergoing programmed cell death, together with mononuclear cells within the periphery, contribute to the inflammatory mechanisms present in T2DM (Kristiansen and Mandrup-Poulsen, 2005). Additional researchers have indicated that IL-6 not only behaves as a moderator of the immune system but additionally has both primary and secondary effects on glucose metabolism and its regulation through its interaction with cells from adipose tissue, skeletal muscle, pancreas, liver and neuroendocrine system (Bashir et al., 2020). In the rats with induced-DM, TNF- α and IL-6 immunoexpressions were both amplified, which reflects the results of earlier work demonstrating raised TNF- α and IL-6 titers in humans with T2DM. These observations offer evidence that T2DM is frequently preceded by a systemic inflammatory response (Lina et al., 2019). In contrast, TNF- α and IL-6 immunoexpressions were both diminished in the murine cohorts that received EA. TNF- α and IL-6 titers were noted by Altwajry et al. (2021) to be suppressed by EA in subjects with osteoarthritis.

Indicators of inflammation, such as TNF- α and IL-6, have been reported to increase in obese rodents and in those demonstrating resistance to insulin. IL-6 is believed to trigger several glucocorticoid receptors, to exert a paracrine influence on the response of adipose tissue to insulin and to promote serum glucagon levels. TNF α is type of proinflammatory cytokine that represent as antitumorogenic and mainly produced by immune cells (Altwajry et al., 2021, El-Masry et al., 2020b). TNF- α has also been proposed as a factor in the generation of insulin resistance as it may adversely impact both the insulin receptor and the insulin receptor substrate (IRS), thus having an inhibitory effect on the response to insulin cues. Additionally, TNF- α has been noted to enhance the expression of cytokine signal suppressor, which forms complexes with IRS1 and IRS2, causing cellular injury. Consequently, insulin-mediated glucose transfer to adipocytes and muscle cells is affected, therefore exacerbating hyperglycemia (Mardiha et al., 2015).

5. Conclusion

This research offers evidence that EA, derived from strawberry fruit, mitigates against pancreatic injury in the STZ murine model of T2DM. The anti-diabetic properties of EA were demonstrated;

these were ascribed to a range of processes, such as heightening sensitivity to insulin, together with an anti-inflammatory influence encompassing the suppression of signaling pathways associated with IL-6 and TNF- α . These findings imply that EA could be a significant actor in the repair of injury to the pancreatic islets in the STZ-induced rodent model of T2DM. Thus, EA may be an innovative treatment approach for the maintenance of normoglycemia in individuals with T2DM.

6. Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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