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# Grape seed extract as a treatment of diabetic nephropathy in type II-induced diabetic rats

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**ABSTRACT**

Background Grape seeds are considered a raw material with high content of phytochemicals that can be used as a source of dietary supplements with antioxidative properties. grape seeds has showed considerable anti-diabetic activity by inhibiting the activities of amylase and α-glucosidase, improving the function and structure of the pancreas and Langerhans islets and alleviating insulin resistance. Moreover, grape can prevent the development of diabetic complications, such as diabetic nephropathy and diabetic retinopathy Several studies have investigated the role of inflammatory markers (cytokines and chemokines) in diabetes development and its complications. Aim: In this study we aimed to evaluation the possible beneficial effects of grape seed proanthocyanidens extract on the renal damage cause by type-2 diabetes mellitus in the terms of renal inflammatory cytokines and serum cystatin-C associated with induced hyperglycemia in male rats by (HFHCD+STZ-induced T2DM rat model. We targeted the determination the cytokines activity IL-6 and IL-10 in Kidney tissues Lysate and the renal function damage golden marker serum cystatin-C so the traditional function markers were done moreover The hepatic , pancreatic and renal histopathological examinations were performed to evaluate the therapeutic potentials of GSPE on the induced metabolic complications caused by T2DM on the kidney, liver and pancreatic tissues of the HFHCD+STZ-induced T2DM male albino rats model.The progression of T2DM can be slowed or even reversed by making lifestyle changes, such as losing weight, eating a healthy diet, and exercising regularly. However, for many people, medication is also necessary to control Hyperglycaemia. Methods: the experiment, the rats were divided into two main groups including SNC group (standard negative control )/ 8 rats) and HFHCD group (High fate High cholesterol Diet)/ 32 rats) which later divided into 4 groups (positive control ( diabetes ) – orally administrated groups with 200 mg CγWWLSP , 400 mg and 5 mg decalazide ) .

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## Introduction:

### Ⅰ. Diabetes Mellitus:

Diabetes mellitus is a long-term metabolic syndrome caused by the lack of and/or resistance to insulin. Cardiovascular disease, retinopathy, nephropathy, angiopathy, and neuropathy are all complications induced by the excessive glucose levels and protein glycation **(1,2).**

due to this pancreatic dysfunction in insulin secretion and response Both major types of diabetes mellitus contribute greatly to health care cost and mortality due to the high incidence of nephropathy leading to ESRD, and the fact that they are a major cause of dialysis and kidney transplantation **(3).** Epidemiological studies data on diabetes mellitus indicate a concerning projected future for T2DM. In 2019 and According (IDF) diabetes caused 4.2 million deaths; and overall affected nearly 463 million adults aged 20 to 79 years old were living with diabetes.Diabetes was the underlying cause of at least 720 billion USD coast. Furthermore, as 1 in 3 diabetics, or 232 million people went undiagnosed and the true disease burden of T2DM is likely underrepresented **(4)** which makes the disease still a silent killer so far, Previously report by IDF the value of the problem, Chronic kidney disease (CKD) is a prevalent condition in many countries, and it is estimated that over $1 trillion is spent globally on end-stage renal disease (ESRD) care.Diabetic kidney disease DKD develops in approximately 40% of diabetic patients and is a major cause of chronic kidney diseases (CKD) and ESRD worldwide **(5).**

Progression of the Diabetes mellitus makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycaemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region in this condition; adipose tissue promotes IR through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation;so the main drivers of the T2DM epidemic are the global rise in obesity, sedentary lifestyles, high caloric diets and population aging, which have quadrupled the incidence and prevalence of T2DM **(6,7);** The progression of T2DM can be slowed or even reversed by making lifestyle changes, such as losing weight, eating a healthy diet, and exercising regularly However, for many people, medication is also necessary to control *Hyperglycaemia.*

### Ⅱ.Diabetic Nephropathy:

Nephropathy is the strongest predictor of mortality in patients with diabetes, its development involves important inter-individual variations. Genome-wide transcriptome studies and high-throughput technologies indicate the activation of inflammatory signaling pathways and oxidative stress highlighting the role of genetic factors; Evidences suggest that epigenetic mechanisms such as DNA methylation, noncoding RNAs and histone modifications can also play a pivotal role in the pathogenesis of diabetic nephropathy. Accordingly, cytokine TNF-alpha, IL-6 and IL-1 beta gene promoter polymorphisms and modulation in expression have been linked to DN susceptibility in subjects. Dysregulation of local metabolic environment triggered by inflammation and subsequent tissue remodeling may initiate kidney damage **(8)** the metabolic mechanisms involving oxidative/ inflammatory pathways are widely accepted. The clear evidence that a chronic hyperglycemic state triggers oxidative stress and inflammation mediated by altered metabolic pathways in a self-perpetuating cycle, promoting progression of cell injury and of end-stage renal disease **(9).**

Hyperglycemia increases reactive oxygen species (ROS) production and decreases enzymatic and non-enzymatic antioxidants signaling (activities of ROS scavenging enzymes) as well as induced glycation and peroxidation of proteins (vascular walls damage) **(10,11)** Chronic hyperglycemia is the key point of macro and microvascular complications associated with diabetes

mellitus. Excess glucose is responsible for inducing redox imbalance and both systemic and intrarenal inflammation, playing a critical role in the pathogenesis of DKD, The pathogenesis of the disease is complex, multifactorial and not fully elucidated; many factors and mechanisms are involved in the development, progression and clinical outcomes of the disease. Despite the disparate mechanisms involved in renal damage related to diabetes mellitus **(9)** where the immune response and inflammation play a major role .In addition, pro-inflammatory signaling pathways and their downstream products are emerging as new biomarkers and promising therapeutic targets to Increase understanding of DN **(12)** DKD exhibits destructive structural changes such as glomerular basement membrane expansion, loss of podocytes, thickening of mesangial matrix and fusion of foot processes **(12)** Conventionally, it is accepted that renal hemodynamics changes, oxidative stress, inflammatory response, hypoxia and renin-angiotensin-aldosterone system (RAAS) are majorly responsible for the pathogenesis of DKD, renal fibrosis plays the key role. Intensified multifactorial interventions, including RAAS blockades, blood pressure and glucose control, and quitting smoking, help to prevent DKD development and progression **(13).**

Cytokines are a group of pharmacologically active, low molecular weight polypeptides these small secreted proteins (<40 kDa), with autocrine, paracrine and juxtacrine effects which, in a coordinated manner, regulate inflammatory and immune responses with the participation of different cytokine-associated signalling pathways. Cytokines are produced throughout the body by cells of varied embryological origin and, in addition to their immune response regulatory role, exert important pleiotropic actions as cardinal effectors of injury (**14)** The release of pro-inflammatory cytokines will lead to activation of immune cells and production as well as the release of further cytokines. Therefore, in the past when the term “cytokine storm” arose, it explained inflammation as a sudden release of cytokines to upregulate an inflammatory process. However, recent research indicates that a simultaneous release of pro- and anti-inflammatory cytokines are mandatory in any immune response; Cytokines suffer from a somewhat inconsistent nomenclature; they are referred to as interleukins, chemokines, or growth factors among many other names. Cytokines are made up of so-called superfamilies, not necessarily describing common genes, but rather similar structures. Furthermore, different cell populations can produce the same cytokine. The effects of cytokines depend on the targeted cell, making them pleiotropic. Also, different cytokines may have the same effect and are therefore redundant. They may, however, also have a synergistic effect. Finally, they potentially trigger signaling cascades, giving the smallest amounts of protein the chance to be devastating in consequence **(15)** A potential participation of inflammatory cytokines in the pathogenesis of DN was suggested for the first time in 1991 by Hasegawa et al. it is recognized that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes mellitus. In the kidney, both blood-borne cells (mainly monocytes and macrophages) and diverse intrinsic renal cells (endothelial, mesangial, dendritic, and tubular epithelial cells) are able to synthesize inflammatory cytokines Furthermore, these Inflammatory cytokines involved in the pathogenesis of diabetes play a significant role in the development and progression of several renal disorders. Renal cells (endothelial, epithelial, mesangial, and tubular cells) are also capable of synthesizing proinflammatory cytokines such as TNF- , IL-1, and IL-6, and, therefore, these cytokines, acting in a paracrine or autocrine manner, may induce a variety of effects on different renal structures a lot of studies indicate a potential relationship between the elevated levels of this inflammatory cytokine and the development and progression of renal injury in DM **(16).** Also, It has been

reported that circulatory IL-6 level is correlated with microvascular complication in type 1 and type 2 diabetic patients and higher serum IL-6 and sIL-6R concentrations are observed in type 2 diabetic patients with retinopathy compared with non-diabetic subjects; Lei CT et al. found that the circulatory levels of IL-6, sIL-6R and sgp130 are all elevated in DKD patients compared with healthy controls. Moreover, the expression of mIL-6R, sIL-6R and gp130 protein is enhanced in kidney cortex of STZ-induced diabetic mice. These observations indicate that under diabetic status not only in the circulation but also in local kidney, the IL-6 classic (via mIL-6R) and trans-signalling (via sIL-6R) are simultaneously activated, and the increased circulatory sgp130 level might represent a compensatory mechanism to limit the activation of IL-6/sIL-6R pathway **(17)** Experimental studies in animal models of diabetes have showed that IL-6 levels are high in patients with DN in comparison with diabetes mellitus patients without nephropathy In addition, the histopathological analysis of human renal samples by immunohistochemistry has demonstrated an increased expression of mRNA encoding IL-6 in cells infiltrating the mesangium, interstitium, and tubules, with a positive relationship with the severity of mesangial expansion , Other functional and structural abnormalities related to DN and progression of renal damage have been associated with IL-6, including abnormalities in the permeability of glomerular endothelium, expansion of mesangial cells and enhanced expression of fibronectin , and increase in the thickness of the GBM in 2015 Donate-Correa, Javier et al. experimental studies have demonstrated an increase in the mRNA levels of IL-6 in the renal cortex of diabetic rats, which is positively associated with the urinary concentration of this cytokine In addition, in animal models of diabetes, wet kidney weight, a marker of renal hypertrophy and an early phenomenon in kidney involvement in DM, has been reported to be enhanced, which was related to mRNA gene expression levels and urine concentration of this cytokine **(16**). IL-6 not only promotes T-cell activation, B-cell differentiation and the cell population expansion during inflammatory response **(18);** but also plays an important role in insulin resistance, lipid metabolism, mitochondrial activities and other situations (**19**) IL-6 a multifunctional cytokine that regulates the immune response, haemopoiesis, the acute phase response and inflammation. IL-6 is produced by various types of cell and influences various cell types, and has multiple biological activities through its unique receptor system. IL-6 exerts its biological activities through two molecules: IL-6R (IL-6 receptor) and gp130. When IL-6 binds to mIL-6R (membrane-bound form of IL-6R), homodimerization of gp130 is induced and a high-affinity functional receptor complex of IL-6, IL-6R and gp130 is formed . Interestingly, sIL-6R (soluble form of IL-6R) also binds with IL-6, and the IL-6–sIL-6R complex can then form a complex with gp130The homodimerization of receptor complex activates JAKs (Janus kinases) that then phosphorylate tyrosine residues in the cytoplasmic domain of gp130. The gp130-mediated JAK activation by IL-6 triggers two main signalling pathways: the gp130 Tyr759-derived SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase-2)/ERK (extracellular-signal-regulated kinase) MAPK (mitogen-activated protein kinase) pathway and the gp130 YXXQ-mediated JAK/STAT (signal transducer and activator of transcription) pathway. Increased IL-6 levels are observed in several human inflammatory diseases, such as rheumatoid arthritis, Castleman's disease and systemic juvenile idiopathic arthritis. IL-6 is also critically involved in experimentally induced autoimmune diseases **(20)** In a biopsy study, IL-6 mRNA was expressed by glomerular resident cells and interstitial cells in patients with DKD; most cells in the area of mesangial proliferation were strongly stained for IL-6 mRNA, and some positive cells were found in the Kimmelstiel–Wilson nodular lesions.

In the interstitium, some tubules and infiltrating cells were also positively stained for IL-6 mRNA, and the interstitial expression of IL-6 mRNA correlated significantly with the degree of interstitial injury. In another biopsy study in type 2 DM, glomerular basement membrane width was directly correlated with IL-6, and both IL-1 and IL-6 have been shown to be overproduced by interstitial and glomerular cells in diabetes, In streptozotocin diabetes in the rat, renal cortical mRNA expression for TNF-α, IL-1, and IL-6 was 2.4-, 1.2-, and 3.4-fold higher than in nondiabetic rats **(21).**
High glucose stimulates IL-6 generation from various renal resident cells which contributes to cellular and tissue injury **(22; 23).**
High IL-6 level was identified in early stage of type 1 diabetes proposing IL-6 is involved in islet cells impairment. Additionally, an IL-6-inducible autoimmunity-related gene (HIP/PAP) was found to be expressed in the pancreas in patients with type-1 diabetes further indicating a potential correlation between IL-6 and autoimmune diabetes In type 2 diabetes, IL-6 level was increased and associated with atherosclerosis development In vitro IL-6 induced insulin resistance which support its role in type 2 diabetes occurrence, furthermore DN patients showed an elevated serum level of inflammatory cytokines, including IL-6, which positively correlated with the extent of proteinuria Meantime, hyperglycemia can trigger podocytes, mesangial cells, interstitial tissue, and tubules to generate IL-6 which contribute to local and systemic inflammatory process in DN; So Chronic inflammatory process participates in the development of microvascular complications of diabetes **(24)** It is noteworthy that cystatin C (Cys-C) was the first protein to be used in clinical diagnostics. In clinical practice Cyst-C is primarily known as a reliable marker for glomerular filtration in kidney dysfunction Serum Cyst-C levels may be more accurate than the glomerular filtration rate as diagnostic value of renal function recently the concentrations of Cyst-C in serum and urine are used as reliable markers of acute kidney injury **(25);** Cys-C is is an important extracellular inhibitor of cysteine proteases; Cys-C, a non-glycosylated protein that plays pleiotropic roles in human vascular patho-physiology. Clinically, the index of Cys-C is used as a diagnostic parameter to record glomerular filtration rate (GFR) due to its easy detection and lower molecular weight. Previously, researchers have done Meta-analyses to prove that serum Cys-C is a predictor of diabetic nephropathy in diabetic patients; increased serum Cyst-C levels have been well established to account for common complications in diabetic population, such as diabetic kidney disease **(26)** Study of the bioactive components of grape seed extracts was first initiated at the beginning of the 20th century. **Albert Szent-Gyorgyi**, a 1937 Nobel Prize winner discovered flavonoids while working on the segregation of vitamin C terming them “vitamin P”. Subsequently, Professor Jacques Masquelier postulated that because pine bark exhibited ascorbate-like effects, it must contain vitamin Calong with flavonoids, which he designated as “pycnogenols”, a term no longer used by the scientific community except as a trademark for proanthocyanidins extracted from French maritime pine bark **(27).** Grape seeds are considered a raw material with high content of phytochemicals that can be used as a source of dietary supplements with antioxidative properties. According to Sabir et al.**(28),** the seed contains fibers (40%) and oils (10–20%). Also, the seeds contain proteins (11%), carbohydrates (26.43%), phenols (7%) and mineral salts according to Owon MA.**( 29)** El-Hawary et al. reported the presence of flavonoids, proanthocyanidins and phenolics in grape seeds **(30)** Souquet et al. showed that showed that grape seeds had a moisture content of 9.4%, 2.6% ash, 28.2% carbohydrates, 35.5% fibers, 13.3% protein, and 10.5% lipids. Furthermore, GSE contained 11.6 mg gallic acid/g dry seed and 13.6 mg quercetin/g dry seed in terms of total phenolic compounds and flavonoids, respectively , Proanthocyanidins, oligomeric

flavonoids are a class of polyphenols found in many plants. It includes catechin and epicatechin oligomers, as well as their gallic acid esters **(31)** GSE had an antioxidant activity of 89.2% and a half-maximal inhibitory concentration (IC50) of 34.5 g/ml **(30)** Phenolic compound distribution in different parts of grapes was reviewed by **Xia et al. in 2010** , gallic acid and flavanols were mainly present in the seed portion**(32)** ,Grape seeds are rich in monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin and (−)-epicatechin-3-Ogallate, and dimeric, trimeric and tetrameric procyanidins **(33)** The capability of tannins to confine proteins underlies, in part, their protective features **(34)** The phenolic compounds in grape seeds are essentially all flavonoids, particularly, flavan-3-ols (catechin, epicatechin and epicatechin-3-O-gallate monomers) and their polypmers. Flavan-3-ols easily condenses into oligomeric procyanidins and polymeric compounds (condensed tannins). The dimeric procyanidins are often referred as B-series, and the trimeric procyanidins as C-series. Five different dimers (procyanidin B1, B2, B3, B4 and B5) and two trimers (C1 and C2) were identified from grape skin and seeds **(35)** Flavonoids are a class of polyphenolic compounds having significant human health benefits **(36)** chemically, proanthocyanidins are oligo- or polymers of flavan-3-ols which are characterized by a common C6–C3–C6 structure consisting of two aromatic rings linked through a three-carbon chain; the building blocks of proanthocyanidins include catechin (C)/epicatechin (EC), gallocatechin (GC)/epigallocatechin (EGC) and afzelechin (AF)/epiafzelechin (EAF) The respective oligomeric proanthocyanidins (polymerization degree (DP)=2–4) and polymeric proanthocyanidins (DP $\geq$ 5) are called procyanidins, prodelphinidins and propelargonidins, being procyanidins the most abundant in nature **(37)** Luca et al. reported that grape seeds contained high content of proanthocyanidins (3532 mg/100 g) **(38)** Recently its well known The antioxidant power of proanthocyanidins (oligomeric proanthocyanidins) is 20 times greater than vitamin E and 50 times greater than vitamin C. extensive studies have shown that proanthocyanidins is beneficial in many areas of health because of its antioxidant effect to bond with collagen, promoting youthful skin, cell health, elasticity, and flexibility, proanthocyanidins protect the body from sun damage, improve vision, improve flexibility in joints, arteries , and body tissues such as the heart, and to improve blood circulation by strengthening capillaries, arteries, and veins **(39)** furthermore, grape seeds proanthocyanidins extract **(GSPE)** has a variety of potent properties such as anti-tumor, anti-inflammation and anti-oxidant which has potential therapeutic abilities in cancer, diabetic complications and brain injury treatment**(40)** hence proved Pharmacokinetic properties and clinical outcomes of Proanthocyanidins are abundant in dietary fruits, vegetables, nuts, legumes, and grains. Among all Proanthocyanidins sources, grape seeds, sorghum, chocolate, and apples have high concentrations. Based on available studies, proanthocyanidins and proanthocyanidins-rich foods have excellent biological effects against metabolic diseases such as antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, cardioprotective, and neuroprotective effects **(41).** Also several studies have reported many therapeutic properties for GSE such as anticancer **(42)**, anti-bacterial **(43),** antioxidant and free radicals scavenging effects **(44 , 45)** grape seed extract inhibited platelet aggregation **(46)** and anti-inflammatory properties**(47)** Also, **Hwang et al.** in **2009** showed that grape seed extract lowered blood glucose level in diabetic rats**(48)**; where **Gao et al. in 2018** reported that GSPE has a protective effect against DN which is achieved by attenuating the ERS-mediated apoptosis via the Caspase-12 pathway**(40)** ; and Saada et al. showed its radioprotective effect against radiation-induced damages **(40)**

The Increasing evidence supports the concept that Proanthocyanidins can mitigate

the adverse health consequences of obesity or of the consumption of diets rich in fat and carbohydrates through their actions at the gastrointestinal tract; Proanthocyanidins can directly modulate gastrointestinal enzymes that are necessary for lipid and glucose absorption, protects gastrointestinal barrier function, mitigate gastrointestinal inflammation, and restore normal microbiota profiles **(50).**

**Gliclazide** is an oral sulfonylurea anti-hyperglycemic agent used to treat non-insulin dependent diabetes mellitus (NIDDM). Gliclazide improves pancreatic β-cells homeostasis, increases pancreatic insulin secretion and its cellular sensitivity, reduces blood glucose levels circulation, and improve peripheral and systemic IR which is available in market in the form of different brand like **Diamicron ( 51).**

## Material and Methods:

### Chemicals:

Nutritional Facts by Calories and Energy of Different Diet (Normal Diet, HFD, HFHCD) showed in table no.1

Chemicals, suppliers, Instruments and software showed in table no.2

### Animals:

Fourty (Eight-weeks-old) weight-matched adult Wistar Albino male rats' (225±25g) from the experimental animal house, Faculty of Pharmacy, Suez Canal University (Ismailia), Ismailia, Egypt. The experimental animals and procedures were conducted in accordance with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals.

### Experimental design:

### 1-Experimental Induction of Diabetes

1- All animals were acclimatized for one week before starting of the experimental study.
2- **At the beginning of the experiment**, the rats were divided into two main groups including:

A- NC group **(negative control healthy group/ 8 rat)** received ad libitum pure drinking water and standard normal diet (SND) during all the experimental period (12 week), and the NC rats were intraperitoneally (IP) injected with a single equivalent dose of fresh cold 0.1M sodium citrate buffer (pH 4.5) **after four weeks** from the beginning of the experiment.

B- **HFHCD+STZ-induced T2DM rat model (positive control group/32 rat)** was fed ad libitum with a **HFHCD** during all the experimental period (12 week) **,** and the overnight fasted rats were intraperitoneally (IP) injected with a single dose of freshly prepared 40 mg/kg STZ (Sigma-Aldrich, St. Louis, MO, USA); After establishment of the model **.** One week after STZ administration, the overnight fasted blood glucose levels **222.88±11.77 mg/dL** (12.37±0.68 mmol/L) compared to the value of NC group **77.88 ± 6.11 mg/dL** (4.32±0.34 mmol/L), which (glycemic > 200 mg/dL).

**Four weeks after STZ administration** (eight weeks from the beginning of the experiment)**HFHCD+STZ induced T2DM rats** (n = 32) were randomly divided into 4 subgroups (8 rats in each group) **as the following:**

**B.1 Positive control group HFHCD +STZ-induced T2DM group** received **HFHCD** feeding for another four weeks (n = 8).

**B.2 Gliclazide group HFHCD + STZ -induced T2DM group** received HFHCD feeding plus orally administrated with **5 mg Gliclazide /kg of rats/day** for four weeks (n = 8) **co adminstaration**).

**B.3 200 mg/kg GSPE group** received HFHCD feeding plus orally administrated with **200 mg /kg of rats/day** (n = 8) **for** four weeks.

**B.4 400 mg/kg GSPE group** received HFHCD feeding plus orally administrated with 400 mg /kg of rats/day (n = 8) for four weeks.

For each experimental group in our study blood samples were taken to measure the levels of different serum biochemical markers that represente conditions of each group before and after drug administration.

**2.Blood, kidney, Liver, and Pancreas Samples Collection** At the end of the experimental duration (12 week), After 30 days of administration the overnight fasted animals were sacrificed under anesthesia (chloroform) 12 h after the last drug administration .The peripheral blood from the ophthalmic vein was collected into the eppendorf tubes. The blood samples were allowed to clot for approximately 1 h at room temperature, and centrifuged at 3000-5000 rpm for 15 min at 4°C to obtain serum. The serum samples were stored at -20°C until the biochemical evaluations were performed; **serum samples** were used to determine the Effects **of** GSPE 95 % on the levels of fasting glucose, creatinene, uraea, and cystatine-C; and so the levels of inflamatory markers IL-6 , IL-10 measured in Kidney Lysate after preparation; Furthermore, organs sample ( kidny, liver and pancreas) samples were excised and rinsed in ice-cold 0.9% saline then fixed in 10% neutral buffered formalin for histopathological investigations after H&E staing

**Kidney Lysate preparation**

renal cytokines IL-6 and IL-10 detrimination in the renal homogenate lysate as its original site of the problem that we investigate; lysate prepared by excetion of the whool kidnyes and divied to be examind and weigh before homogenization.; kidneys Rinsed in ice-cold **PBS** (Phosphate buffered saline ) PH 7.4 to remove excess blood thoroughly; to obtain 10% wt/vol homogenates; **PBS** were prepared by (800 mL DW, 8g NaCl ,200 mg KCl, 1.44 g Na2HPO4, 245 mg KH2PO4 andjusting to desired PH 7.4 and compled to 1 L by DW) Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with the homogenizer the homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant.which collected (lysate) to a fresh 1.5 ml eppendorf tube labbled with sample No and tracked in a table of samples then stord in -80 to be Measured

**3. Serum Biochemical Parameters.** Determination of the Effects of 200 mg/kg GSPE, 400 mg/kg GSPE, and Gliclazide on :
1-The Level of Blood Fasting Glucose.
2-The Levels of creatinene
3-The Levels of Blood uraea
4-The Level of serum cystatine-C(cys-C).
5-The Level of renal IL-6
6-The Level of renal IL-10

**4. Histopathological Examinations**
Kidney, Liver and Pancreas Tissue Specimens. Partial tissue of rat kidney, liver and pancreas was collected, fixed with 4% formaldehyde solution, and gradually dehydrated with a gradient of 75%, 80%, 95%, and 100% ethanol. Next, the tissue was embedded in paraffin, and sections with 3 μm thickness were cut using a microtome. After the xylene dewaxing treatment, the sections were washed with absolute ethanol, followed by washing with distilled water for 2 min. Next, the sections were stained with hematoxylin for 5 min, washed with tap water for 1 min, subjected to hydrochloric acid-ethanol differentiation for 15 sec, soaked in warm water at 50°C for 3 min, stained with eosin for 1 min, and dehydrated with gradient ethanol and xylene medium. They were sealed with dry gum when they were dried and, subsequently, observed under a ×400 microscope (M165FC; Leica) to observe the pathological structure of rat kidneys. The cytoplasm was stained pink, and the nuclei were stained blue. The use of thin microscopic slices allowed the continuity of anatomical structures to be determined with certainty **(52).** A semiquantitative index was used to evaluate the degree of tissue damage from 0 to 4, where 0 represents no lesion, and 1, 2, 3 and 4 represents completely damage **(53).**

**5. Statistical Analysis.**

**Evaluation of the Effect of HFHCD Feeding plus STZ Injection on the Level of Blood Glucose after One Week from STZ Administration (after Five Weeks from the Experimental Duration)**

**1-** As shown in **Figure 1,** after five weeks from the experimental study, the

HFHCD+STZ-induced hyperglycemia rats introduced a significant ($p < 0.05$) increase in the levels of serum fasting glucose compared to the Normal Control group rats (12.37 ± 0.68 vs. 4.32 ± 0.34 mmol/L).

**Evaluation of the Effects of the Effects of 200 mg/kg GSPE, 400 mg/kg GSPE, and 5mg/kg Gliclazide on the Levels and Activities of Different Biochemical Parameters of the HFHCD+STZ-Induced T2DM Rat Model**

2- **Serum Urea (mg/dl)**

**Figure 2** show multi-comparison between groups (n=8) by LSD according to urea (mg/dl) and describe the post-treatment values of urea, significant increase in untreated diabetic group copmared with healthy group with P value < 0.001, Urea showed significant improvment in 200 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001 while significant improvment in 400 mg/kg GSPE treated group compared with 200 mg/kg GSPE treated group with P value< 0.002 ; significant improvement in Gliclazide treated group compared with untreated diabetic group with P value < 0.001; no significant increase in 400 mg/kg GSPE treated group compared with Gliclazide treated group with P value = 0.531

**3- Serum Creatinine (mg/dl)**

**Figures 3** show multi-comparison between groups (n=8) by LSD according to Creatinine (mg/dl) and describe the post-treatment values of Creatinine, Creatinine showed highly significant increase in untreated diabetic group compared with healthy group with P value < 0.001; significant improvment in 400mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001 while Creatinine showed no significant increase in 400 mg/kg GSPE treated group compared with normal healthy group with P value = 0.362; significant improvement in Creatinine in Gliclazide treated group compared with untreated diabetic group with P value < 0.001

**4- serum Cys -C (ng/ml)**

**Figures-4** show LSD for multi-comparison between groups (n=8) according to Cys -C (ng/ml), Cys-C showed significant increase in untreated diabetic group compared with normal healthy group with P value < 0.001; Cys-C showed significant improvment in 200 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; Cys-C showed significant improvement in 400 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; Cys-C showed no significant increase in 400 mg/kg GSPE treated group compared with normal healthy group with P value = 0.790; Cys-C showed highly significant increase in untreated diabetic group compared with Gliclazide treated group with P value < 0.001; Cys-C showed no significant increase in Gliclazide treated group compared with normal healthy group with P value= 0.804; Cys-C showed no significant increase in 400 mg/kg GSPE treated group compared with normal healthy group with P value = 0.790

**5- kidney lysate IL 6 (pg/ml)**

**Figures 5** show multi-comparison between groups (n=8) by LSD according to IL 6 (pg/ml) and describe the post-treatment values, IL-6 proinflamatory cytokine showed highly significant increase in untreated diabetic group compared with healthy group With P value < 0.001; significant improvement in 200 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; significant improvement in 400 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; IL-6 have no significant increase in 400 mg/kg GSPE treated group compared with normal healthy group with P value = 0.080; significant improvement of IL-6 in Gliclazide treated group compared with untreated diabetic group with P value < 0.001.

**6- kidney lysate IL-10 (pg/ml)**

**Figures 6** show multi-comparison between groups (n=8) by LSD according to IL 10 (pg/ml) and describe the post-treatment values, IL-10 showed significant increase in untreated diabetic group compared with normal healthy group with P value < 0.001; significant improvement in 200 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; significant improvement in 400 mg/kg GSPE treated group compared with 200 mg/kg GSPE treated group with P value = 0.002; IL-10 showed no significant increase in 400 mg/kg GSPE treated group compared with normal healthy group with P value = 0.682 which is the sign normal state due to the power of the dose; IL-10 showed highly significant increase in untreated diabetic group compared with Gliclazide treated group with P value < 0.001

**At the end of the experimental study**, after 12 week of administration rats showed a significant amelioration in their pro-inflamatory cytokines IL-6 , retardation in antiinflamatory cytokines IL-10 were observed which indicat the passway in upregulation in their antioxidant antidiabetic antiinflamatory defense mechanisms within the different treated induced experimental rat group compared with untreated rats groups.

**Using Pearson correlation coefficients test Pearson Correlation Standardized Coefficient Relationships between Several Serum Biochemical Variable Parameters (post-treatment values) within the Experimental Rat Groups (Simple Linear Regression Analysis / Linear Curve Fit Estimation Model )**

**Figures 7** show strength of the Pearson correlation standardized coefficient relationships between each two different serum biochemical variable parameters (**Creatinine, Cys C, IL6, IL10**) within the experimental rat groups. As shown in Figures **7** a very strong positive correlation coefficients between **Cys C** and **Creatinine** levels with highly statistically significant correlation, means that the higher **Cys C**, the higher the **Creatinine** will be where the correlation coefficient was 0.877 with a significance of 0.004 . Also, there is a strong positive correlation between **IL6** and **IL10** levels with statistically significant correlation, means that the higher **IL6**, the higher the **IL10** will be where the correlation coefficient was 0.833 with a significance of 0.010 among **Gliclazide treated group** ; also a **very strong positive correlation** coefficients between **IL6** and **Creatinine** levels with highly statistically significant correlation, means that the higher **IL6**, the higher the **Creatinine** will be where the value of correlation coefficient was 0.732 with a significance of 0.039; the presence of strong positive bivariate relationships between each two different serum biochemical variable parameters (**Creatinine** and **Cys C**; so **IL6** and **IL10**; **IL6** and **Creatinine** )within different experimental groups were observed.

Under pathological conditions, high proinflamatory cytokine level were accompanied by a greatly increase in the levels of serum **Creatinine**, Cys- C and **IL10;** and decrease in the level of serum **Creatinine, Cys- C, IL6, IL10** within the different experimental rat groups. After treatment, upregulate the function of pancreatic tissue observed in the histopathological examination and improvement their kidney markers( urea, Creatinine and Cys-C **)** were also related to retardation the levels of renal proinflamatory cytokine **IL6**, the response of anti-inflamatory cytokine **IL10**, and elevated level of renal **IL10** within the different induced experimental rat groups.

## Histological changes

**Histopathological Evaluations of Pancreas Tissue Specimens show that:**

**Figure 8**, Histopathological evaluations of **Pancreas** (H&E, X400), tissue specimens of the different experimental rat groups,

where The normal healthy rats showed normal pancreaticacini, pancreatic islets, and pancreatic duct with **(Lesion Score: 0);** where the Pancreas of HFHCD+STZ indued T2DM of untreated Group in Figure 8 (A untreated T2DM) specimens showed diffuse hyperplastic pancreatic islets (*) and multi-focal degeneration of the pancreatic acini (arrows), with **(Lesion Score: +++)** and Figure 8 (B untreated T2DM) showed severely hyperplastic pancreatic ducts (*) with **(Lesion Score: +++);** the Pancreas of HFHCD+STZ indued T2DM of Gliclazide treated Group in Figure 8 (A Gliclazide treated T2DM) showed multi-focal hyperplastic pancreatic islets (*) with absence of degeneration of the pancreatic acini with **(Lesion Score: ++)** and the Pancreas of HFHCD+STZ indued T2DM of **Gliclazide** treated Group in Figure 8 (B Gliclazide treated T2DM) showed moderately hyperplastic pancreatic duct (*) with **(Lesion Score: +);** the Pancreas of HFHCD+STZ indued T2DM of 200 mg/kg GSPE treated Group in Figure 8 (A 200 mg/kg GSPE treated T2DM) showed multi-focal hyperplastic pancreatic islets **(*)** with absence of degeneration of the pancreatic acini, with **(Lesion Score: ++)** and in Figure 8 (B 200 mg/kg GSPE treated T2DM) showed showing severe interstitial blood vessels congestion (arrow head), together with mononuclear cells infiltration (*), and pancreatic duct hyperplasia (arrow) with **(Lesion Score:+++);** the Pancreas of HFHCD+STZ indued T2DM of 400 mg/kg GSPE treated Group in Figure 8 (A 400 mg/kg GSPE treated T2DM) showed focal hyperplastic pancreatic islets (*) with absence of degeneration of the pancreatic acini, with **(Lesion Score: + )** and in Figure 8 (B 400 mg/kg GSPE treated T2DM) showed mild congestion in the interstitial blood vessels (*), there is no hyperplasia in the pancreatic ducts with **(Lesion Score: + )**

**Histopathological Evaluations of liver Tissue Specimens show that:**

**Figure 9**, Histopathological evaluations of liver (H&E, X400) tissue specimens of the different experimental rat groups. Figure 9 The normal healthy rats showed normal hepatic parenchyma with polyhedral hepatocytes, normal blood sinusoids, and normal portal tract with (Lesion Score: 0); where the liver of HFHCD+STZ indued T2DM of untreated Group in Figure 9 (A untreated T2DM) specimens showed multiple severe inflammatory portal lesions; note the congestion of hepatoportal blood vessel (arrow head) and the leucocytic cells infiltration (arrows) with **(Lesion Score: +++)** and Figure 9 (B untreated T2DM) showed diffuse multi-focal areas of necrotic hepatocytes disintegrated and infiltrated with mononuclear inflammatory cells with **(Lesion Score: +++)**; the liver of HFHCD+STZ indued T2DM of Gliclazide treated Group in Figure 9 (A Gliclazide treated T2DM) showed mild inflammatory portal lesions; note the congestion of hepatoportal blood vessel (arrow head) and the leucocytic cells infiltration (arrow) with (Lesion Score: +) and the liver of HFHCD+STZ indued T2DM of Gliclazide treated Group in Figure 9 (B Gliclazide treated T2DM) showed congested central vein (*) with dilated blood sinusoids (arrows) with apparently healthy hepatocytes without necrosis or mononuclear cells infiltrations with (Lesion Score: +); the liver of HFHCD+STZ indued T2DM of 200 mg/kg GSPE treated Group in Figure 9 (A 200 mg/kg GSPE treated T2DM) showed moderate inflammatory portal lesions; note the congestion of hepatoportal blood vessel (arrow head) and the leucocytic cells infiltration (arrow) with (Lesion Score: ++) and in Figure 9 (B 200 mg/kg GSPE treated T2DM) showed multi-focal areas of necrotic hepatocytes disintegrated and infiltrated with mononuclear inflammatory cells with (Lesion Score: ++); the liver of HFHCD + STZ indued T2DM of 400 mg/kg GSPE treated Group in Figure 9 (A 400 mg/kg GSPE treated T2DM) showed no inflammatory portal lesions with healthy hepatoportal blood vessels and bile duct(Lesion Score: 0 ) and in Figure 9 (B

400 mg/kg GSPE treated T2DM) showed apparently healthy hepatocytes without necrosis or mononuclear cells infiltrations with (Lesion Score: 0 )

**Histopathological Evaluations of kideny Tissue Specimens show that:**

**Figure 10**, Histopathological evaluations of Kidney (H&E, X400), tissue specimens of the different experimental rat groups. The normal healthy rats showed normal renal glomeruli and renal tubules with **(Lesion Score: 0);** where the Kidney of HFHCD+STZ indued T2DM of untreated Group in Figure 10 (A untreated T2DM) specimens showed multi-focal congestion of the interstitial blood vessels with thick muscle wall (arrow) and peri vascular edema (*),with **(Lesion Score: +++)** and Figure 10 (B untreated T2DM) showed diffuse glomerular epithelial vacuolation (arrows) and diffuse renal tubules degeneration (*), **(Lesion Score: +++);** the Kidney of HFHCD+STZ indued T2DM of Gliclazide treated Group in Figure 10 (A Gliclazide treated T2DM) showing focal congestion of the interstitial blood vessels (arrow head) with no thick muscle wall or peri vascular edema, with **(Lesion Score: ++)** and the Kidney of HFHCD+STZ indued T2DM of Gliclazide treated Group in Figure 10 (B Gliclazide treated T2DM) showing focal glomerular epithelial vacuolation (arrows) with no renal tubules degeneration, with **(Lesion Score: ++) ;** the Kidney of HFHCD+STZ indued T2DM of 200 mg/kg GSPE treated Group in Figure 10 (A 200 mg/kg GSPE treated T2DM) showed multi-focal moderate congestion of the glomerular blood capillaries (arrows) and of the interstitial blood vessels with thick muscle wall (arrow head) and perivascular hemorrhage (*), with **(Lesion Score: ++)** and in Figure 10 (B 200 mg/kg GSPE treated T2DM) showed focal glomerular epithelial vacuolation (arrows) with no renal tubules degeneration, with **(Lesion Score: +);** the Kidney of HFHCD+STZ indued T2DM of 400 mg/kg GSPE treated Group in Figure 10 (A 400 mg/kg GSPE treated T2DM) showed slight congestion of the interstitial blood vessels with slight thick muscle wall (*) with absence of the perivascular edema or hemorrhage, with **(Lesion Score: +).** and in Figure 10 (B 400 mg/kg GSPE treated T2DM) showed apparently healthy renal glomeruli and renal tubules,with **(Lesion Score: 0).**

## Discussion

Diabetes is a progressive chronic multi-factorial disorder that arises from insulin deficiency (type I diabetes) and/or insulin resistance (type II diabetes). However, it is characterized by chronic high blood glucose levels that results from defects in the carbohydrate metabolism, leading to failure and dysfunction of many body organs such as liver, kidney and eye **(54)** Which, currently effects on >220 million people worldwide, and expects to effect on 400 million by 2030; Coronary artery disease, atherosclerosis, hypertension, hyperlipidemia, nephropathy, retinopathy, and neuropathy are considered as the most serious IR-induced diabetic complications. In diabetes, the elevated levels of glucose and highly increase production of the inflammatory cytokines that develop cellular injury and progress chronic diabetic complications by generating reactive oxygen species (ROS) and attenuating the antioxidant signaling via glycation of the antioxidant enzymes **(55)**

**In our study**, we first established a model of type 2 diabetes; The establishment of a T2DM model can be induced with HFHCD+STZ indued T2DM, a high-fat diet for a period of time to induce insulin resistance, and then a small dose of intraperitoneal injection of STZ is given to destroy the islets, resulting in insulin secretion deficiency-induced hyperglycemia disease **(67).**

Controlling glycemia is a practical approach to the prevention and treatment of diabetes mellitus. Some natural products can lower the level of postprandial blood glucose by inhibiting the activities of amylase and α-glucosidase, which are important enzymes

with respect to delaying the hydrolysis of complex sugars and decreasing the release of glucosyl units into the blood **(56).**

In recent years, phytochemicals generate a lot of attention due to their health benefits which necessitate more scientific studies **(57) In our study** the histopathological improvments in pancreas, Langerhans islets and liver tissues was in accordance with a study by Irak et al. revealed that grape seed extract prevented histopathological changes of pancreas and improved the function and structure of the pancreas and Langerhans islets in rat with streptozotocin-induced diabetes **(58).** Insulin resistance is also an important pathogenic factor with respect to diabetes mellitus, which is caused by the low sensitivity of peripheral tissue to insulin. It was reported that virgin grape seed oil significantly alleviated insulin resistance in high-fat-diet-fed mice; the study results further suggest that polyphenols might be the most essential factor regulating insulin resistance **(59) Recent study reported that:** HFHCD feeding induced and developed asymptomatic hepatic steatosis, chronic liver injury, and inflammatory and sever fibrotic (hepatic collagen fibers accumulation) features in the liver histological sections of the HFHCD+STZ-induced diabetic complications mice model **(68) Our results in this study in concerned with Liver,** the histological sections of administrated group by (400 mg/kg/day GSPE) for 30 days was Like the appearance of normal control ones where in **Figure 9** (A 400 mg/kg GSPE) reported that Liver showing apparently healthy hepatocytes without necrosis or mononuclear cells infiltrations, with **(Lesion Score: 0);** in the histological sections Figure 9 (B 400 mg/kg GSPE) that reported that is no inflammatory portal lesions with healthy hepatoportal blood vessels and bile duct with **(Score lesion: 0**) while **induced untreated diabetic** showed multiple severe inflammatory portal lesions and note the congestion of hepatoportal blood vessel, leucocytic cells infiltration **Figure 9 (A untreated diabetic)** with **( Lesion Score:+++)**

Grape seeds Proanthocyanidins are secondary metabolites formed from a combination of flavanol monomers; they are the most abundant plant phenolic compounds, second only to lignin and they constitute the second major phenolic compound in the human diet. **Qi et al.** reported Proanthocyanidins are named according to the size of the polymer, with dimers, trimers, and tetramers usually termed oligomers and those composed of more than five sub-units called multimers. Among these, dimers are the most widely distributed, researched, and relevant, and the simplest types of proanthocyanidins primarily comprise catechin and epicatechin homo- or heterodimers **(59)** According to Sabir et al. Also**,** Grape seeds are considered a raw material with high content of phytochemicals that can be used as a source of dietary supplements with antioxidative properties **(28)** El-Hawary et al. reported the presence of flavonoids, proanthocyanidins and phenolics in grape seeds; GSE contained 11.6 mg gallic acid/g dry seed and 13.6 mg quercetin/g dry seed in terms of total phenolic compounds and flavonoids, respectively. GSE had an antioxidant activity of 89.2% and a half-maximal inhibitory concentration (IC50) of 34.5 g/ml **(30).**

Grape seed proanthocyanidin extract also reduced renal damage in rats with diabetes mellitus by ameliorating oxidative-stress-mediated injury by activating the Nrf2 signaling pathway **(60).**

Also several studies have reported many therapeutic properties for GSE such as anticancer **(42),** anti-bacterial power **(43),** antioxidant and free radicals scavenging effects **(44) (45)** grape seed extract inhibited platelet aggregation **(46)** and anti-inflammatory properties **(47)** Also, **Hwang et al. in 2009** showed that grape seed extract lowered blood glucose level in diabetic rats **(48);** where **Gao et al. in 2018** reported that GSPE has a protective effect against DN which is achieved by attenuating the ERS mediated apoptosis via the Caspase 12 pathway(**40)**; and Saada et al. showed its radioprotective effect against radiation-

induced damages **(40)** serum cystatin C has been reported not only as a marker of glomerular filtration rate (GFR), but also as an independent risk factor for all-cause and cardiovascular mortality among elderly persons with chronic kidney disease (CKD) or without renal impairment that it is synthesized and secreted by all nucleated cells in every human tissue, including kidney, liver, pancreas, intestine. Almost every organ of the body can express Cys-C due to its high concentration in biologic fluids **(69;70)** Diagnostic utility of serum cystatin C (CysC) in normoalbuminuric patients has also been well documented in a cohort of T1DM, indicating the predictive performance of CysC before the renal dysfunction appears **(71).** In multiple studies done among CKD patients with T1DM and T2DM, showed a significant role of serum CysC as a predictor of progression to ESRD **(72)** these studies reported that and **Our results in this study in concerned with kidney** inflammatory revealed a significant improvements in the post-treatment values of IL-6 proinflamatory cytokine in kidneny homogenate of (HFHCD+STZ-induced T2DM rats) where a significant improvement in 200 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001; **Also** a significant improvement in 400 mg/kg GSPE treated group compared with untreated diabetic group with P value < 0.001 where the biochemical markers of the function showed a highly significant improvments in values of urea and creatinine of in **400 mg/kg/day GSPE** treated group for 30 days when compared with **positive untreated diabetic group** with **P < 0.002;** a highly significant improvement in serum **Cys-C** of induced T2DM rats administrated by **400 mg/kg/day GSPE** for 30 days when compared with induced T2DM rats **positive untreated diabetic group** with **P < 0.001 where** highly significant increase **of** serum **Cys-C** in **untreated diabetic group** compared with **normal healthy group** with **P value < 0.001**

The earliest kidney changes that can be seen in the HFHCD+STZ IP T2DM model are glomerular hypertrophy and mesangial expansion. These changes are caused by the increased blood glucose levels that are associated with T2DM. The increased blood glucose levels damage the glomeruli, the small blood vessels in the kidneys that filter waste products from the blood. This damage leads to the hypertrophy (enlargement) of the glomeruli and the expansion of the mesangium, the tissue that supports the glomeruli **(75,76)** Our results were in accordance with study of Gao et al. on HFHCD+STZ-induced T2DM rats that concluded that Grape seed proanthocyanidins protect against streptozotocin-Induced diabetic nephropathy by attenuating endoplasmic reticulum stress-Induced apoptosis **(40)** the histopathology results also showed that the GSPE had very little glomerular damage and so concluded **(74) Our results in this study in concerned with Kidney** the histological sections of administrated group by **(400 mg/kg/day GSPE)** for 30 days was **(A 400 mg/kg GSPE treated T2DM**) showed a significant improvments that only slight congestion of the interstitial blood vessels with slight thick muscle wall with absence of the perivascular edema or hemorrhage, with **(Lesion Score: +) and** in (**B 400 mg/kg GSPE** treated T2DM) showed apparently healthy renal glomeruli and renal tubules , with **(Lesion Score: 0)** where the Kidney of HFHCD +STZ indued T2DM of untreated Group in **(A untreated T2DM)** specimens showed multi-focal congestion of the interstitial blood vessels with thick muscle wall and peri vascular edema ,with **(Lesion Score: +++)** and **(B untreated T2DM)** showed diffuse glomerular epithelial vacuolation and diffuse renal tubules degeneration, **(Lesion Score: +++)**

Grape seed proanthocyanidin has showed considerable anti-diabetic activity by inhibiting the activities of amylase and α-glucosidase, improving the function and structure of the pancreas and langerhans islets and alleviating insulin resistance;

Moreover, Grape seed proanthocyanidin can prevent the development of diabetic complications, such as diabetic nephropathy and diabetic retinopathy several studies have investigated the role of inflammatory markers (cytokines and chemokines) in diabetes development and its complications. These researches were expected to shed light on the processes that underpin the disease's origin and progression. Increased secretion of pro-inflammatory cytokines has been linked to severe damage in pancreas of diabetic experimental rats **(62).** Moreover, Toll-like receptor 4 (TLR-4) was reported to be involved in diabetes progression and insulin deficiency and/or resistance **(63). In** recent years, phytochemicals generate a lot of attention due to their health benefits which necessitate more scientific studies **(61)** Mahmoud et al. showed that untreated diabetic animals have a high level of TLR-4 and linked this to diabetes progression and pancreatic damage. One decade ago, the therapeutic potential of Mesenchymal stem cells have been transplanted, paving the way for insulin-secreting pancreatic cell regeneration using stem cells **(64).** MSCs have the potential to regenerate and differentiate into specialized cells such as adipocytes, chondrocytes, myocytes and osteoblasts when given the correct conditions and signals **(65). Insulin** producing cells (MSCs) for treating diabetes pathology and its repercussions has been extensively researched **(62).** Although thousands of plants have been used both traditionally and medically, only a few plant extracts have been proven to have a specific mechanism of action on MSCs. In the presence of a specific plant extract, MSC differentiation into specific lineage-committed progenitor can help expand fields for regenerative medicine and therapy. As a result, the researches study emphasizes the significance of bioactive chemicals derived from GSE in MSCs proliferation and differentiation in vitro and in vivo. Extract from different plants usually contains bioactive components like polyphenol, flavonoid, and other chemicals which are beneficial as a therapy for infectious and chronic disorders, the Plant extract has been reported to increase the proliferation of stem cells and has an anticancer effect. **(2)(66).**
Natural products such as vitamin D3, green tea, Aloe vera and GSE activate bone marrow stem cell development; also, oleic and linoleic acids activate the growth of hematopoietic stem cell **(73).**

**Conclusion:**

**In conclusion**, the HFHCD feeding for continuous 12 week plus IP injection with a single dosage of 40 mg/kg STZ were developed a HFHCD+STZ-induced T2DM rat model.
In this study, the HFHCD+STZ-induced T2DM rat model was characterized by degeneration, infiltration, and necrotic features in pancrease, liver and kidney changes as well as increasing hyperglycemia, elevating the level of proinflamatory cytokine IL-6,and creatine and the Cys-C compared to the Normal healthy group.
GSPE reduces renal ischemia/reperfusion injuries in rats, the anti-oxidant capability and anti inflammatory effect of GSPE was proven too from the decrease in inflammatory interleukines concentration in the kidny homogenates compared with untreated induced diabetic group rats in our study resuls; the histopathological examination revealed normal liver, kidney pancreatic architecture with no evidence of inflammation compared by the negative control untreated group. This indicated the efficacy of GSPE in reducing the oxidative stress and inflammation that results from diabetes induction, which allowed islets of Langerhans regeneration; this astonishing power of GSPE lies in its phytochemical components (Flavonoids and phenolic compounds).**Our findings indicated** too that GSPE has renoprotective effect properties via modulating the secretion of pro-inflammatory cytokines.

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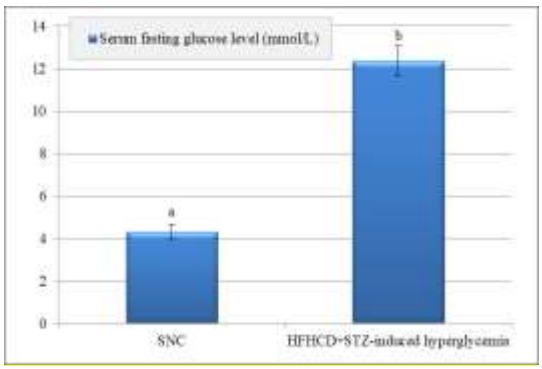



**Figure 1** - Evaluation of the Effect of HFHCD Feeding plus STZ Injection on the Level of Blood Glucose after One Week from STZ Administration (after Five Weeks from the Experimental Duration)

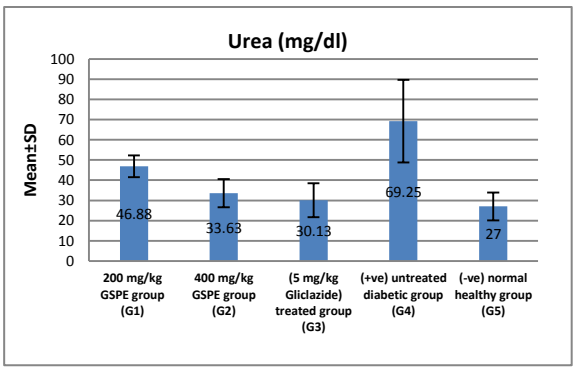



**Figure 2**- show that Urea (mg/dl) distribution among the studied groups

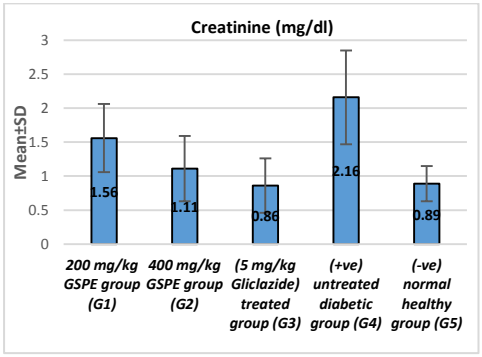



**Figure 3: show that Creatinine (mg/dl) distribution among the studied groups**

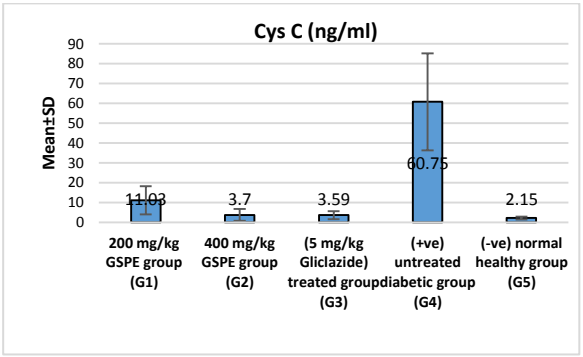



**Figure 4 - Cys-C (ng/ml) distribution among the studied groups**

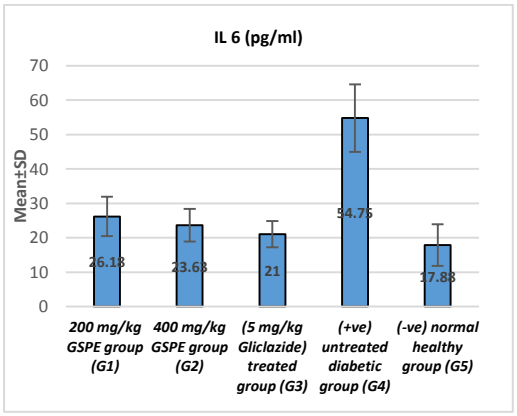



**Figure 5- show that IL-6 (pg/ml) distribution among the studied groups**

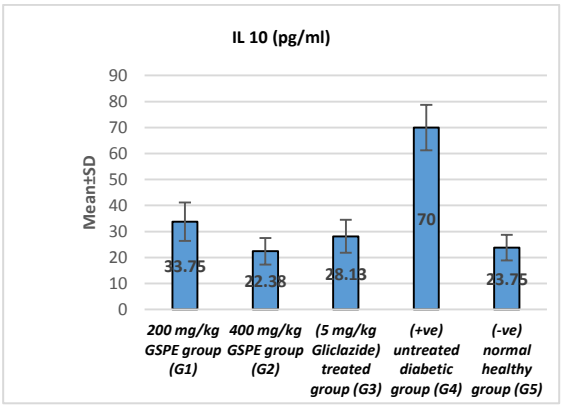
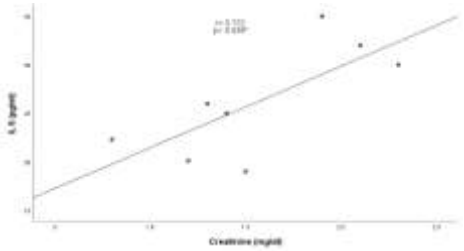
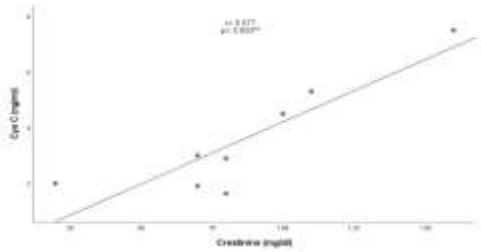


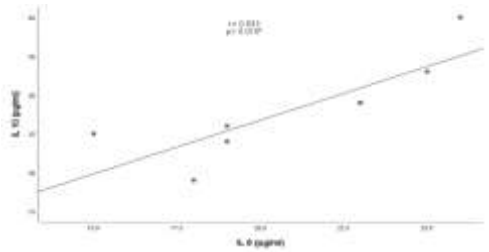

**Figure 6 - show that IL10 (pg/ml) distribution among the studied groups**



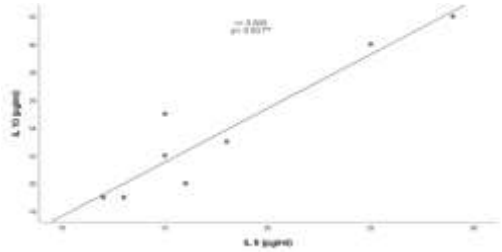
**Scatter dots showing distribution between IL-6 & Creatinine among 200 mg/kg/day GSPE treated group**



**Scatter dots showing distribution between Cys C & Creatinine among Gliclazide treated group**

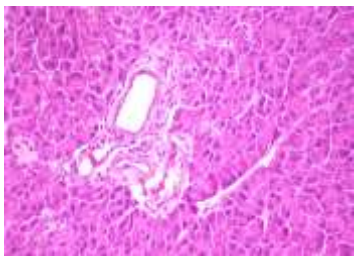


Scatter dots showing distribution between IL6 & IL10 among Gliclazide treated group

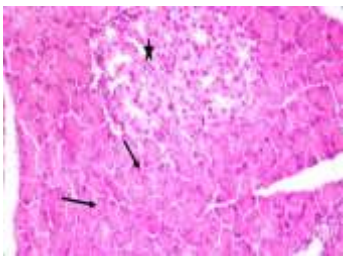


Scatter dots showing distribution between IL6 & IL10 among normal healthy group

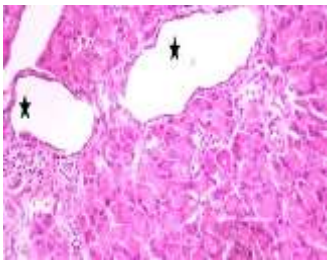
**Figure 7**



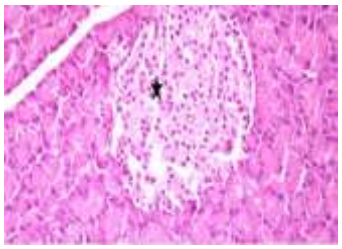
normal healthy



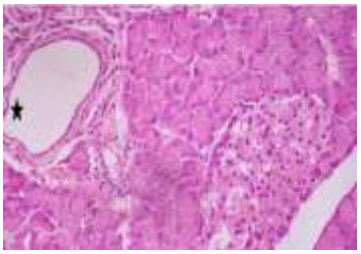
A untreated diabetic



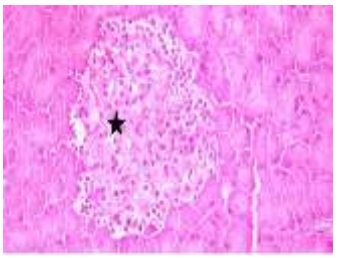
B untreated diabetic



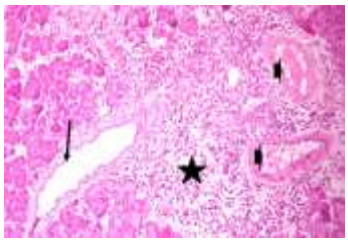
A Gliclazide



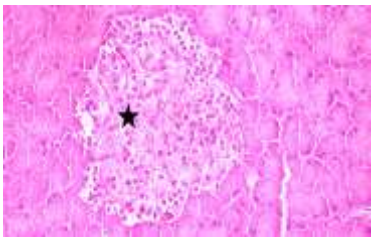
B Gliclazide



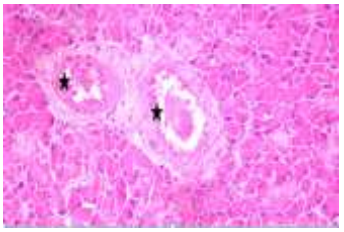
A 200 mg/kg GSPE



B 200 mg/kg GSPE



A 400 mg/kg GSPE



B 400 mg/kg GSPE

**Figure: 8 Histopathological Examination Of Pancreas Biopsy**

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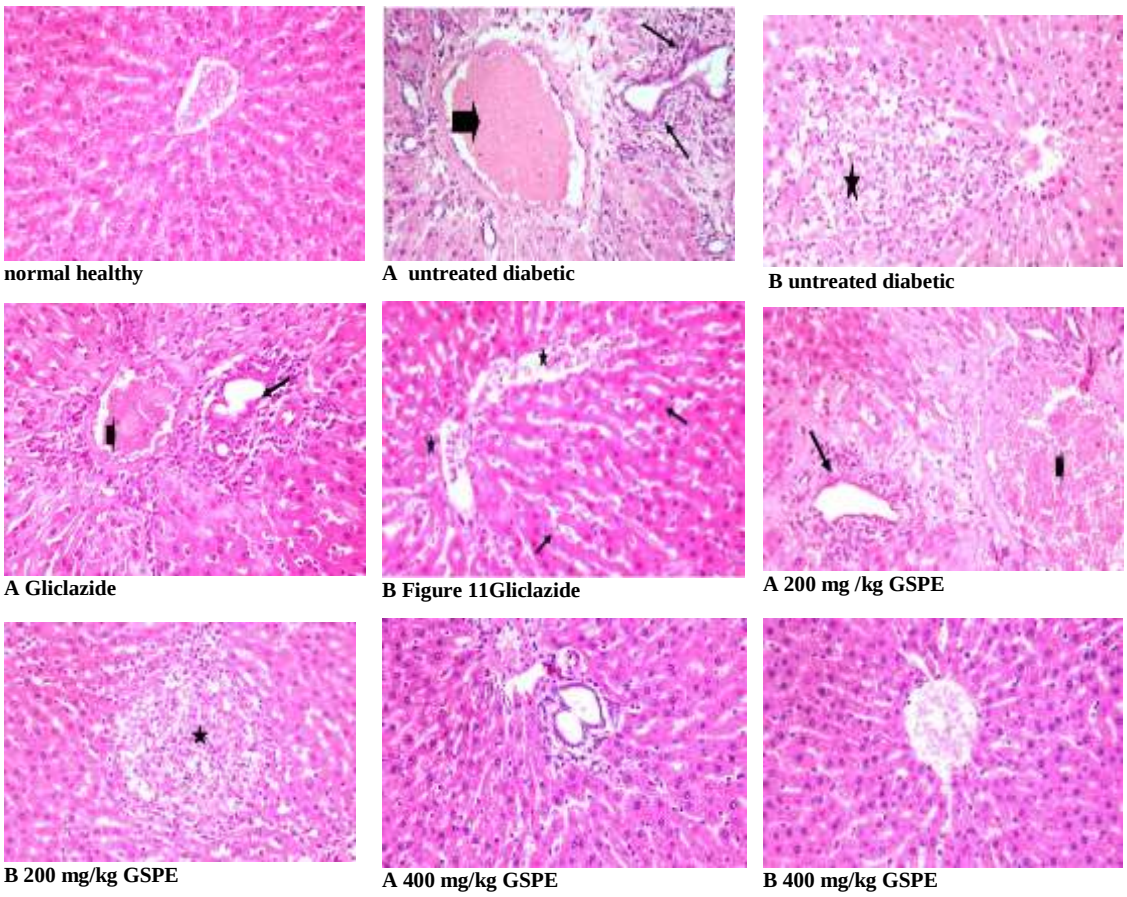


**Figure :9 histopathological examination of liver biopsy**

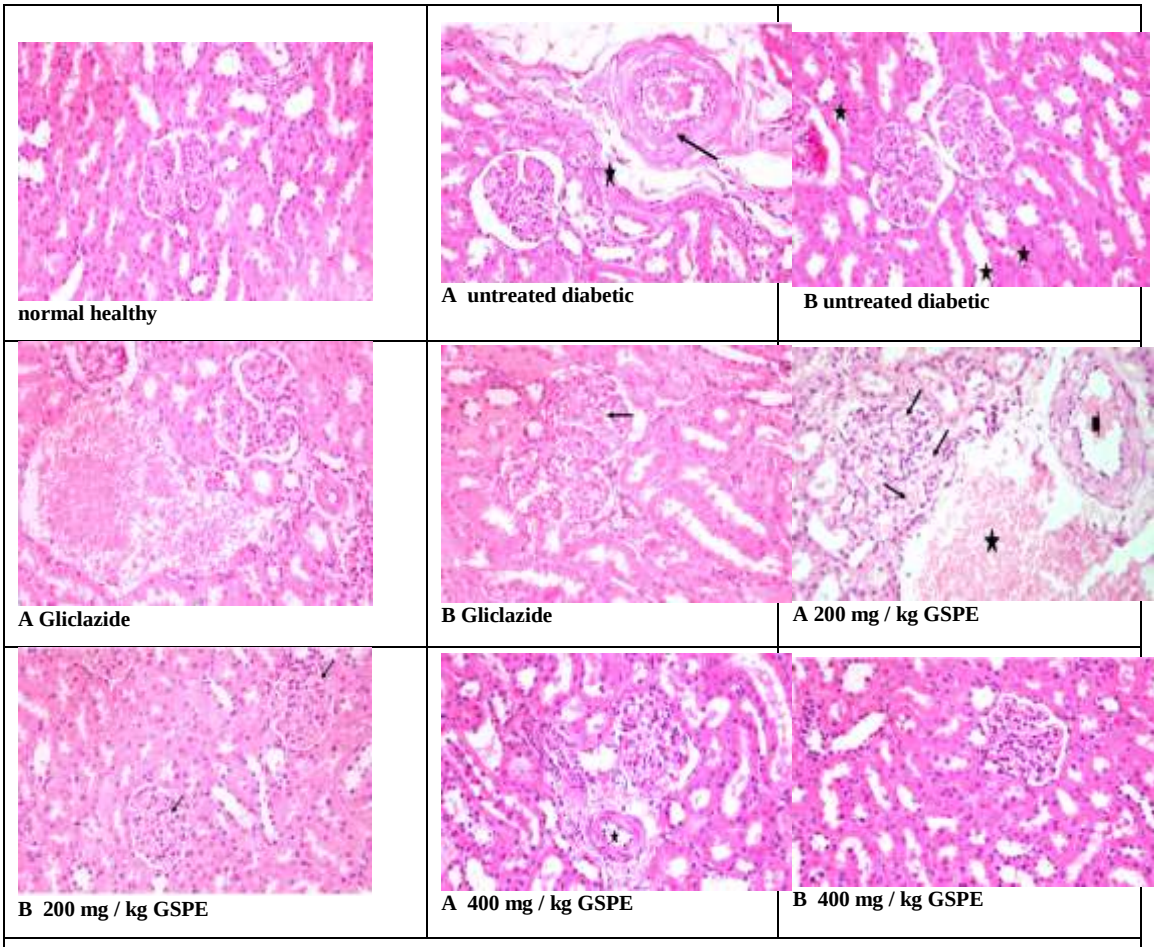


**Figure 10: Histopathological Examination Of Kidneys Biopsy**

**Table 1: Nutritional Facts by Calories and Energy of Different Diet (Normal Diet, HFD, HFHCD) (77; 78; 79)**

| | Normal Diet (ND) | High Fat Diet (HFD) | High Fat High Cholesterol Diet (HFHCD) |
|---|---|---|---|
| K Cal / 100 g | 270 | 521 | 453 |
| Energy from fat,% | 10 | 60 | 40 |
| Energy from carbohaydrates, % | 70 | 20 | 40 |
| Energy from protein, % | 20 | 20 | 20 |
| Cholesterol per 100 g | -- | -- | 1.25 : 1.5 g |

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**Table . 2 List of chemicals, kits, suppliers, instruments, and softwares**.

| ***Chemicals*** | ***Sources*** |
|---|---|
| 0.9% saline. | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Chloroform | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Ethanol (95%). | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Methanol (95%). | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Ultrapure water (deionized water). | Millipore Corp., Billerica, MA, USA. |
| Ethylenediaminetetraacetic acid (EDTA). | Sigma-Aldrich, St. Louis, MO, USA |
| Isopropanol (2-propanol). | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Formaldehyde/ Formalin (37%). | ADWIC, El-Nasr Pharmaceutical Chemicals Co., Egypt. |
| Standard normal diet (SND). | 2016, Teklad Custom Diet, Envigo, Gannat, France. |
| Grape Seed Extract 95% OPC (Proanthocyanidins) powder | NuSci Brand HerbStoreUSA,Walnut,California,USA |
| Streptozotocin (STZ). | Sigma-Aldrich, St. Louis, MO, USA |
| DIAMICRON MR 60 mg (Gliclazide). | Servier, Zuellig Pharma, Egypt. |
| **Kits** | Sources |
| Creatinine (CREA) | Human Diagnostics Worldwide, Egypt. |
| UREA/BUN - COLOR | Human Diagnostics Worldwide, Egypt. |
| Rat Interleukin 6 (IL-6) ELISA Kit | (BT lab)Shanghai Korain Biotech Co., Ltd |
| Rat Interleukin 6 (IL-10) ELISA Kit | (BT lab)Shanghai Korain Biotech Co., Ltd |
| Rat cystatine –c (cys-c) ELISA Kit | (BT lab)Shanghai Korain Biotech Co., Ltd |
| **Instruments** | ***Sources*** |
| RIELE Photometer 5010 V5 Plus | ROBERT *RIELE* GmbH & Co (Germany) |
| ELISA HumaReader HS | Human gmbha (Germany) |
| Refrigerated centrifuge | Thermo Fisher Scientific inc. (USA) |
| Thermo Fisher Ultra-Low Freezer | Thermo Fisher Scientific inc. (USA) |
| Tissue Homogeniser Kinematica™ Polytron™ PT2500E Desktop Homogenizer | Thermo Fisher Scientific inc. (USA) |
| pH meter. | Linaton, Cambridge, UK. |
| Water bath. | Clifton, England. |
| Weight balance. | Précis, Switterland. |
| (light microscope M165 FC ; Leica) | Leica Biosystems |
| glucometer Accu-ChekActive | **Roche** Diagnostics Corporation**,** USA Corporation, Indianapolis,USA |
| **Software** | |
| One way ANOVA with LSD, IBM SPSS 26.0 | SPSS, Inc., Chicago, IL, USA. |