OPEN ACCESS

GAVIN PUBLISHERS

Cytology & Histology Reports

Massoud AMA, et al. Cytol Histol Rep 2: 107.

Research Article

DOI: 10.29011/CHR-107.100007

Possible Therapeutic Role of Fermented Deglycyrrhizinizated Liquorice Extract on Experimentally Induced Diabetic Keratopathy in Rats. Histological Study

Ahmad Mohamed Ali Massoud[1], Faika Hassan El Ebiary[2], Hadwa Ali Abd Al- khalek[2], Sara Abdel Gawad[2]

[1]Departments of Tropical Medicine, Faculty of Medicine Al- Azhar University Cairo, Egypt

[2]Departments of Histology & Cell Biology, Faculty of Medicine, Ain-Shams University Cairo, Egypt

[*]**Corresponding author:** Sara Abdel Gawad Elsebay, Faculty of Medicine, Department of Histology and Cell Biology, Ain Shams University, Cairo, Egypt. Tel:+201067800614; Email: Sara_elsebaey@med.asu.edu.eg

Citation: Massoud AMA, El Ebiary FH, Al- khalek HAA, Gawad SA (2019) Possible Therapeutic Role of Fermented Deglycyrrhizinizated Liquorice Extract on Experimentally Induced Diabetic Keratopathy in Rats. Histological Study. Cytol Histol Rep 2: 107. DOI: 10.29011/CHR-107.100007

Received Date: 23 April 2019; **Accepted Date:** 30 April 2019; **Published Date:** 8 May, 2019

Abstract

Introduction: Long-term hyperglycemia has toxic effects on all cells in the body, and its most profound effects on eye tissues are on the cornea and retina. Seventy percent of diabetic patients are complicated by keratopathy, including recurrent corneal epithelial erosions, delayed wound healing, ulcers and edema. Glycyrrhiza glabra; also called Liquorice; is a well-known medicinal plant used in traditional medicine for its pharmacological value. However, excess consumption of Liquorice can lead to hypertension. Therefore, Deglycyrrhizinated Liquorice extract (DGL) is mostly used to avoid the hypertensive side effects of the glycyrrhizin in whole liquorice. Moreover, fermentation of Liquorice can produce excess amounts of amylase and lipase beside a spectrum of flavonoids naturally occurring in liquorice, hence the newly innovated Fermented Deglycyrrhizinized Liquorice (FDGL) was used in this study.

Aim: In the present study we aimed to investigate the potential therapeutic effects of FDGL, as a natural product, on experimentally induced diabetic keratopathy in rats.

Materials and Methods: Forty adult male albino rats were divided into three groups. Group I: Control animals, which were divided into citrate buffer treated and FDGL treated subgroups (10 rats each). Group II (diabetic) and Group III (diabetic and FDGL treated), 10 rats each. At the end of experiment, the rats were sacrificed and the corneas of different groups were processed for light and electron microscopic examination. The thickness of corneal layers was measured by image analyser and statistical analysis was done.

Results: Light microscopic examination of the cornea of diabetic group showed numerous cells with darkly stained nuclei together with focal sloughing of the surface epithelial layers. Also there was a significant decrease of mean corneal thickness, comparable to the control group. Transmission electron microscopic examination of the cornea of the diabetic group revealed ill-defined hemidesmosomal junctions with interruption of the desmosomal junctions. The stroma showed focal loss of collagen fibrils. Diabetic and FDGL treated group (Group III) showed amelioration of the effects of diabetes on the structure of the cornea.

Conclusion: The data obtained from the present study revealed that FDGL has a potential ameliorating effect on diabetes mellitus induced structural changes in the cornea.

Keywords: Cornea; Diabetes Mellitus; Histology; Streptozotocin

Introduction

Diabetes Mellitus (DM) is a metabolic disease characterized by elevated blood glucose with increase the probability of both macrovascular and microvascular complications [1]. DM is a global epidemic that shows worldwide increase in the prevalence with subsequent increase in the morbidity and mortality in all forms of DM [2]. With the rapid increase in the incidence of DM worldwide, ocular complications have become a leading cause of blindness. In addition to the abnormalities of the retina (retinopathy) and the lens (cataract), different forms of corneal disorders, collectively termed Diabetic Keratopathy (DK), are also relatively common in diabetic patients [3]. Plants are considered as a major source of medicine in all cultures from ancient times. Glycyrrhiza glabra; also called Liquorice; is a well-known medicinal plant used in traditional medicine for its pharmacological value [4]. The therapeutic properties of Liquorice are well documented since the Ancient Egyptian times. The roots of Liquorice are the most used parts whereas leaves are considered as an agrochemical waste. Nutritionally, liquorice is a source of proteins, amino acids, polysaccharides and simple sugars, mineral salts such as sodium, calcium, potassium, copper and zinc, pectins, starches, sterols and gums [5]. Liquorice contains a large number of biological compounds, mostly triterpenes, saponins (responsible for the sweet taste), and flavonoids [6]. However, the contents of these compounds may differ significantly according to the geographic sources affecting the therapeutic properties of liquorice [5].

Currently, liquorice extracts are used in pharmaceutical and food industries, as well as in the manufacture of functional foods and food supplements. Indeed, the most important industrial use of liquorice is the production of food additives, such as flavours and sweetening agents [5]. However, excess consumption of liquorice can lead to the classic symptoms of hypertension, potassium loss and muscular weakness. Therefore, Deglycyrrhizinated Liquorice extract (DGL) is most commonly used to avoid the hypertensive side effects of the glycyrrhetinic acid in whole liquorice [7]. Diabetic patients (both type 1 and 2) suffer from a drop in their serum amylase level proportional with the severity of their diabetic complication [8]. So Liquorice was fermented under specific circumstances to produce large amounts of enzymes as amylase and lipase. The whole formulation of the drug was specified and termed as Fermented Deglycyrrhizinized Liquorice extract (FDGL) [9]. With the rapidly increasing prevalence of DM worldwide, there is a great need for effective and safe function biomaterials with anti-diabetic criteria. Therefore, in the present study we aimed to investigate the therapeutic potential of FDGL, as a natural product, on the cornea of STZ induced diabetic male albino rats.

Materials and Methods

The experiment was performed in the Medical Research Center, Faculty of Medicine, Ain Shams University, Cairo, Egypt. The laboratory animals were treated in accordance with the Institutional Animal Ethics Committee of Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Drug formulation

The drug powder was prepared in the Pharmacology Research Unit, National Research Center, Cairo, Egypt, according to the European Patent Specification Ep 1 925 312 B1 [9], through 3 consecutive steps: fermentation of liquorice root, deglycyrrhizination and lyophilization.

Preliminary pilot study was conducted on adult rats of Sprague Dawley strain to detect the acute toxicity (LD50) of FDGL extract in rats according to Buck *et al*. [10] in the Pharmacology Research Unit- National Research Center. It was concluded that FDGL extract is safe for oral administration. Another preliminary pilot study was performed on adult Sprague Dawley rats to detect the chronic toxicity of FDGL extract according to Afifi *et al*. [11] in Pharmacology Research Unit-National Research Center. It was also deduced that FDGL extract is safe for oral administration up to 0.12 gm/kg body weight /day for 2 months.

Animal grouping

Forty adult male albino rats weighing 180-200 grams were used in this study. Rats were given food and tap water ad libitum. They were divided randomly into three groups:

Group I (Control group): Included 20 rats and were further subdivided into:

Subgroup Ia

Included 10 rats. Each animal received single intraperitoneal injection of 0.5 ml of 0.1 M/L citrate buffer (Vehicle of STZ) and were sacrificed after four weeks.

Subgroup Ib

Included 10 rats. Each rat received FDGL at a dose of 0.12 gm/kg body weight /day, by oral gavage for 2 weeks then animals were sacrificed.

Group II (Diabetic group)

Included 10 rats. The rats were fasted for 18 hours prior to the induction of DM. Diabetes mellitus was induced by a single I.P. injection of freshly prepared solution of STZ (Sigma, USA) at dose of 40 mg/kg body weight diluted in 0.5 ml of 0.1M/L citrate buffer, pH 4.5 [12]. Diabetes was confirmed by measuring the blood glucose level 3 days after the induction. Rats were considered to be diabetic with serum glucose level > 250 mg/dL [13]. The animals were sacrificed 4 weeks after the induction of DM.

Group III (Diabetic and FDGL treated group)

Included 10 rats. Induction of diabetes was done as in group II. After confirmation of diabetes, rats were left for two weeks then FDGL was given daily for further two weeks as in subgroup Ib, then they were sacrificed (i.e. after four weeks from confirmation of diabetes).

Blood glucose level analysis

A drop of fresh blood was collected from the animal`s tail using a lancet at fasting conditions. Blood glucose levels were measured in all groups twice weekly using glucometer instrument (Accua-check, ROCHE, Germany).

Histological study

For light microscopic examination; both eye balls were enucleated in dim light. One eye was fixed for three minutes in 40% formalin and then was cut vertically into two halves and was immersed in 10% neutral-buffered formalin, dehydrated, cleared and embedded in paraffin. Sections of the eye ball (5 µm thickness) were stained with Haematoxylin and Eosin (H&E). Photographs were taken from the mid cornea. For Transmission Electron Microscope (TEM) examination; the cornea of the other eye was separated from the posterior segment (including retina). They were divided into small pieces ($1mm^3$) and fixed at 4 °C in phosphate buffered gluteraldehyde. Ultrathin sections (50-60 nm) were stained with uranyl acetate and lead citrate [14]. Sections were examined by TEM (JEOL 1200 EXII) at the Faculty of Medicine, El Azhar University.

Morphometric measurements

Samples were analyzed using an image Leica Q win V.3 program installed on a computer in the Histology and Cell Biology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope with built-in camera (Leica Microsystems GmbH, Ernst-Leitz-StraBe, Wetzlar, Germany). Ten specimens from ten different rats of each group were examined (n=10). From each specimen, five different captured non-overlapping fields were taken. Five different readings from every captured photo were counted and the mean was calculated for each specimen. Measurements were taken by an independent observer blinded to the specimens' details so as to perform an unbiased assessment.

The following parameters were measured

- The mean of the thickness of the corneal epithelial layer.
- The mean of the total cornea thickness.

All the measurements were taken at the mid cornea and at high-power fields of magnification (×400).

Statistical analysis

The measured parameters as well as the mean of blood glucose level were collected, revised and subjected to statistical analysis using one-way analysis of variance performed with SPSS.21 program (IBM Inc., Chicago, Illinois, USA) Analysis for Variance (ANOVA)-one-way analysis and post-Hoc Least Significant Difference (LSD). The significance of the data was determined by the P value. P values greater than 0.05 were considered non-significant, and P values less than 0.05 were considered significant. Summary of the data was expressed as mean ± Standard Deviation (SD).

Results

Light microscopic results of the cornea

Examination of H&E stained corneal sections of the control subgroup Ia showed the layers of the stratified squamous non keratinized epithelium of the cornea. These layers are: the basal layer formed of columnar cells, intermediate layers formed of polygonal cells and superficial layers formed of squamous cells. The corneal epithelium appeared resting on a regular basement membrane (Bowman's membrane). The main thickness of cornea was the substantia propria which appeared formed of regularly arranged collagen fibers with flattened corneal stromal cells in between. Descemet's membrane appeared next to the substantia propria and it was covered by flattened Descemet's endothelium (Figure 1). The histological structure of control subgroup Ib was more or less comparable to that of the control subgroup Ia.

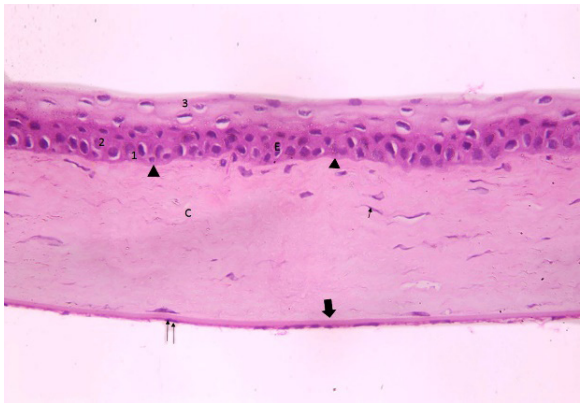

Figure 1: A photomicrograph of a section of cornea of Group I (subgroup Ia) showing the stratified squamous non-keratinized epithelium (E) resting on regular Bowman's membrane (arrow head). Notice the layers of the epithelium, (1) basal layer, (2) intermediate polygonal layers, (3) squamous cells of superficial layers. The substantia propria contains regularly arranged collagen bundles (C) and flattened corneal stromal cells (short arrows) squeezed in between. The inner surface of the cornea is lined by a single flattened Descemet's endothelial cell layer (↑↑) supported by Descemet's membrane (thick arrow) H&E X640.

In the diabetic group (Group II), focal structural changes were noted. Some superficial epithelial cells revealed dark nuclei while others were desquamated with lightly stained nuclei. Numerous dark nuclei were seen in the basal and intermediate cell layers. Some epithelial cells showed vacuolated cytoplasm. Areas of reduced thickness of corneal epithelium were evident. There was sub-epithelial vascularization. The substantia propria showed areas of widely spaced collagen fibers. The Descemet's membrane appeared relatively thick and wavy, as compared to that of the control group (Figure 2a,2b).

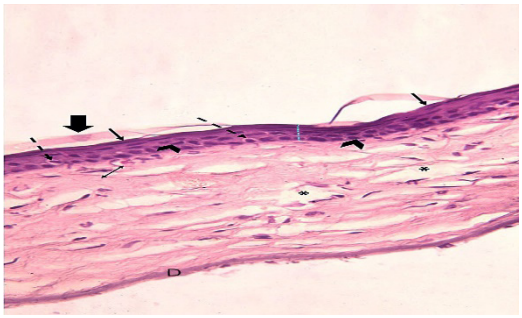

Figure 2a: A photomicrograph of a section of cornea of Group II showing some of the surface epithelial cells with darkly stained nuclei (thin arrows) and others appear desquamated with light stained nuclei (thick arrow). Multiple dark nuclei (arrowheads) are seen in the basal and intermediate layers. Some epithelial cells show vacuolated cytoplasm (dashed arrow). An apparent decrease in thickness of corneal epithelium is seen (dashed line). Sub-epithelial invasion of blood vessels (↕) is noticed. The substantia propria shows wide separation of its collagen fibers (*). The Descemet's membrane appears relatively thick and wavy (D) H&E X640.

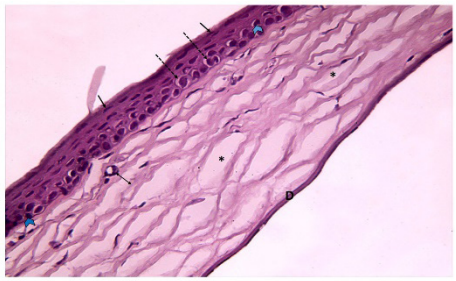

Figure 2b: A photomicrograph of a section of cornea of Group II showing some of the surface epithelial cells with darkly stained nuclei (thin arrows). Multiple dark nuclei (arrowheads) are seen in the basal and intermediate layers. Few epithelial cells show vacuolated cytoplasm (dashed arrow). Subepithelial invasion of blood vessels (↕) is noticed. The substantia propria shows wide separation of its collagen fibers (*). The Descemet's membrane appears relatively thick (D) H&E X640.

Meanwhile, examination of H&E stained sections of Group III (diabetic and FDGL treated group) revealed that the histological profile of the corneal epithelium, the substantia propria, Descemet's membrane and Descemet's endothelium were nearly similar to that of the control group except for focal minimal separation between collagen fibers of the substantia propria (Figure 3).

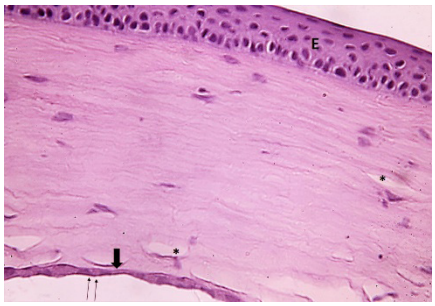

Figure 3: A photomicrograph of a section of cornea of Group III showing normal appearance of corneal epithelium (E). Minimal focal separation of collagen fibers of corneal stroma could be seen (*). Notice Descemet's membrane (thick arrow) and the endothelium (↑↑) H&E X640.

Transmission electron microscopic results of the cornea

Electron microscopic examination of the corneal sections of the control subgroup Ia revealed the corneal stratified squamous non keratinized epithelium resting on regular basement membrane (Bowman's membrane) with apparent hemidesmosomes. The cells of the basal layer of corneal epithelium appeared with oval euchromatic nuclei and their cytoplasm showed mitochondria (Figure 4).

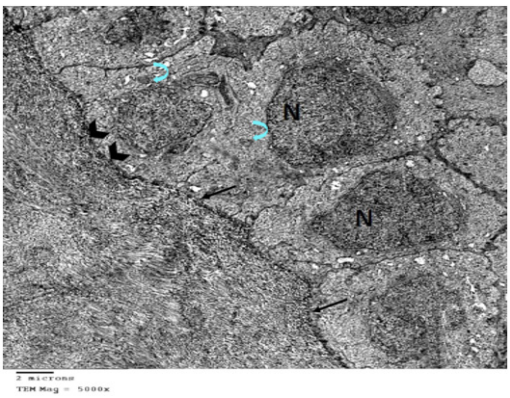

Figure 4: An electron micrograph of a section of cornea of Group I (subgroup Ia) showing cells of the basal layer of the corneal epithelium with euchromatic nuclei (N) and lying on regular basement membrane (↑). The cytoplasm showing mitochondria (curved arrow). Notice the hemidesmosomal junctions (arrowheads) TEM X5000.

Cells of the intermediate layers of corneal epithelium showed rounded euchromatic nuclei. The cells were connected with desmosomal junction (Figure 5). The top layer of corneal epithelium was formed of cells with flattened nuclei and microvilli projecting from their apical surfaces (Figure 6). The substantia propria was seen formed of regular lamellae of collagen fibrils running in longitudinal and transverse directions with flattened stromal cells squeezed in between (Figure 7).

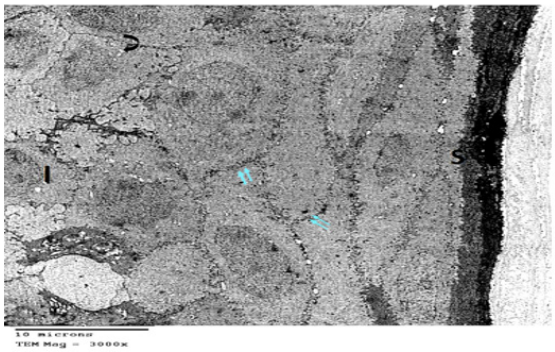

Figure 5: An electron micrograph of a section of cornea of Group I (subgroup Ia) showing the intermediate polygonal (I) and superficial epithelial cells (s). The cytoplasm of epithelial cells showing mitochondria (curved arrow). Notice the presence of desmosomes in between the epithelial cells (↑↑) TEM X3000.

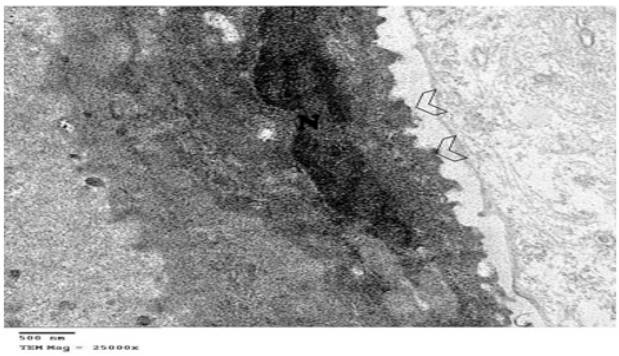

Figure 6: An electron micrograph of a section of cornea of Group I (subgroup Ia) showing the nuclei of superficial epithelial cells (N). The microvilli appear projecting from these cells (arrowheads) TEM X25000.

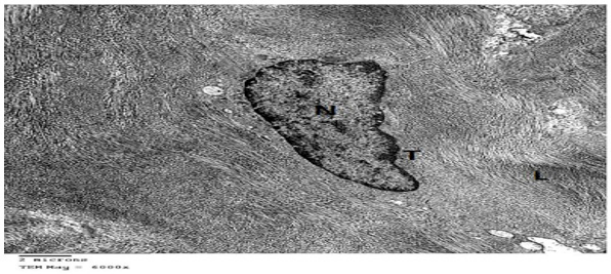

Figure 7: An electron micrograph of a section of cornea of Group I (subgroup Ia) showing the nucleus of the stromal cells (N) in between regular collagen fibrils lamellae running in longitudinal (L) and transverse (T) directions TEM X6000.

Descemet's membrane appeared as a homogenous non cellular layer beneath which the Descemet's endothelium. The cytoplasm of Descemet's endothelium showed pinocytotic vesicles (Figure 8). The histological structure of the control subgroup Ib was more or less comparable to that of the control subgroup Ia. In the diabetic group (Group II), corneal sections showed the corneal epithelium resting on discontinuous basement membrane with ill-defined hemidesmosomes. The basal cells showed irregularly shaped folded nuclei, cytoplasmic vacuoles and degenerated mitochondria (Figure 9).

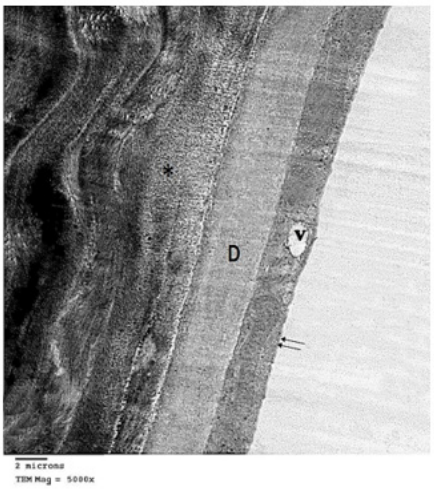

Figure 8: An electron micrograph of a section of cornea of Group I (subgroup Ia) showing homogenous Descemet's membrane (D), endothelial cells (↑↑) with their pinocytotic vesicles (V). Notice a part of corneal stroma (*) TEM X5000.

Figure 9: An electron micrograph of a section of cornea of Group II showing cells of the basal layer of corneal epithelium lying on basement membrane with ill-defined hemidesmosomes (↑). Notice the presence of irregularly shaped folded nuclei (N), cytoplasmic vacuoles (thick arrow) and degenerated mitochondria (M) inside the cytoplasm of the epithelial cells. The stroma shows focal separation of its collagen fibrils (*). There is focal increase of collagen fibrils of Bowman's layer underneath basement membrane (triangle) TEM X5000.

Cells of the intermediate and superficial layers of corneal epithelium revealed degenerated mitochondria and the nuclei of cells of the middle layer appeared with corrugated nuclear membrane. The desmosomal junctions between the cells were

interrupted (Figure 10). The most superficial cells showed distorted microvilli projecting from their apical surfaces (Figure 11). The stromal layers showed areas of fragmentation and disruption of their collagen fibrils (Figure 12). Descemet's membrane appeared thick with the presence of electron dense areas. Endothelial cells were distorted with irregular nuclei. Their cytoplasm revealed numerous large vacuoles as well as vacuolated mitochondria (Figure 13).

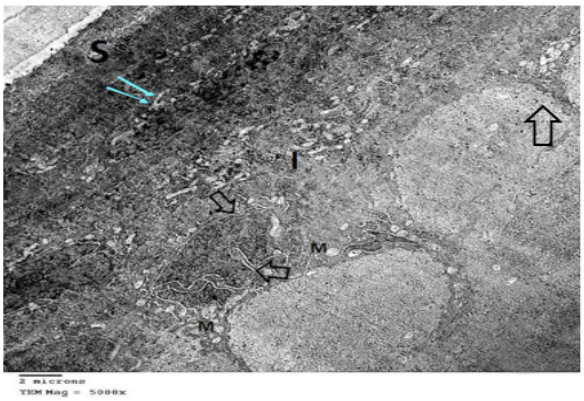

Figure 10: An electron micrograph of a section of cornea of Group II showing the cells of intermediate (I) and superficial (S) layers of corneal epithelium which revealed degenerated mitochondria (M). The nuclei of cells of middle layer showing corrugated nuclear membrane (thick arrow). Areas of interrupted desmosomal junctions are seen (↑↑) TEM X5000.

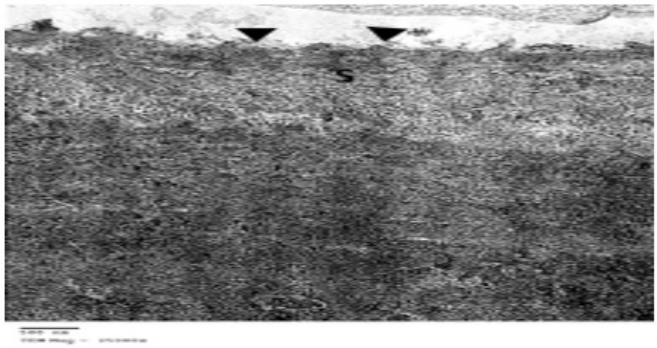

Figure 11: An electron micrograph of a section of cornea of Group II showing the upper most cells of the corneal epithelium (S) which appear with distorted stunted microvilli (▼) TEM X25000.

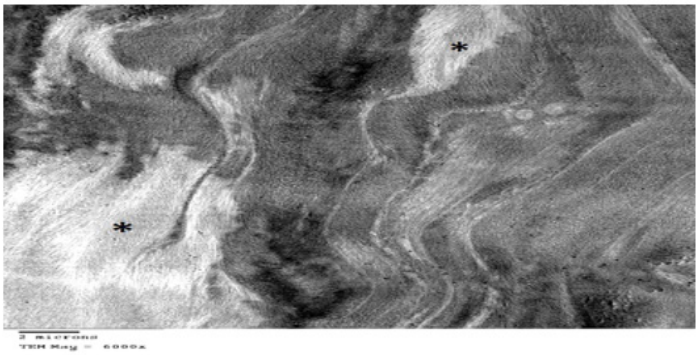

Figure 12: An electron micrograph of a section of cornea of Group II showing areas of disruption of collagen fibrils (*) in corneal stroma TEM X6000.

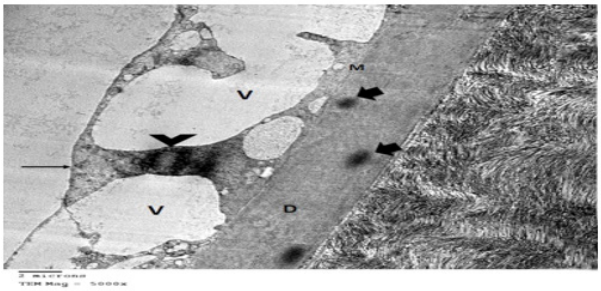

Figure 13: An electron micrograph of a section of cornea of Group II showing the Descemet's membrane (D) with electron dense areas (thick arrow). Endothelial cell (↑) appears distorted with abnormal nucleus (arrowhead) and its cytoplasm shows multiple large vacuoles (V) and vacuolated mitochondria (M) TEM X5000.

The histological structure of the cornea of Group III (diabetic and FDGL treated group) was comparable to that of the control. The Bowman's membrane of the corneal epithelium appeared continuous with well apparent hemidesmosomes (Figure 14). The desmosomes between the epithelial cells appeared intact (Figure 15). The superficial cells of corneal epithelium showing numerous intact microvilli (Figure 16). The construction of most of the substantia propria appeared all most similar to that of the control group except for focal areas with minimal separation of collagen fibrils (Figure 17) Descemet's membrane appeared homogenous and thickened. Descemet's endothelial cells are of normal shape and size with cytoplasmic pinocytic vesicles (Figure 18).

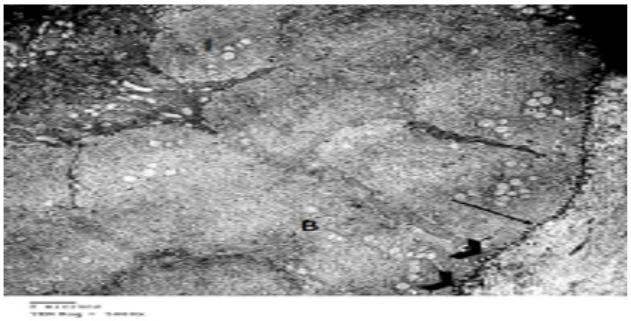

Figure 14: An electron micrograph of a section of cornea of Group III showing normal appearance of corneal epithelial cells of basal (B) and intermediate layers (I). Notice continuous Bowman's membrane (↑) and apparent hemidesmosomes (arrowheads) TEM X5000.

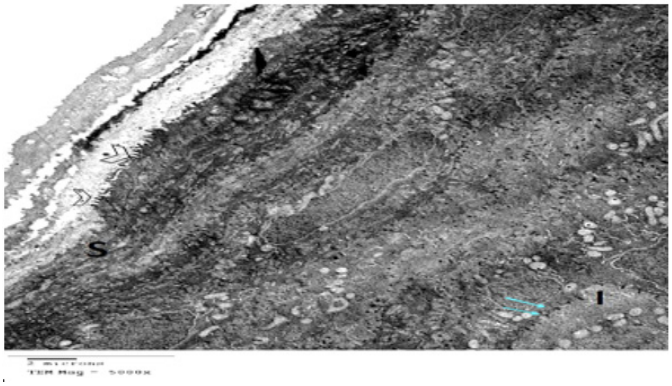

Figure 15: An electron micrograph a section of cornea of Group III showing intermediate (I) and superficial (S) layers of the corneal epithelium. The superficial cells reveal intact microvilli (arrowheads). Notice well developed desmosomal junctions (↑↑) between corneal epithelial cells TEM X5000.

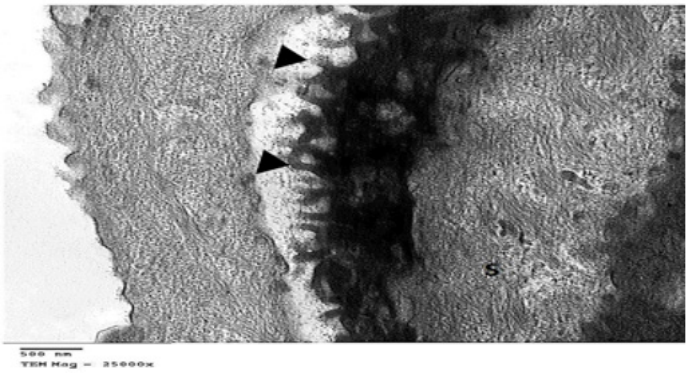

Figure 16: An electron micrograph of a section of cornea of Group III showing the upper most cells of the corneal epithelium (S) which appear with intact microvilli (▼) TEM X25000.

Figure 17: An electron micrograph of a section of cornea of Group III showing a Stromal Cell (SC) squeezed in between the collagen fibrils lamellae. Notice the normal appearance of corneal stroma with longitudinal (L) and transverse (T) fibrils. Notice the focal areas of minimal separation of collagen fibrilis (*) TEM X5000.

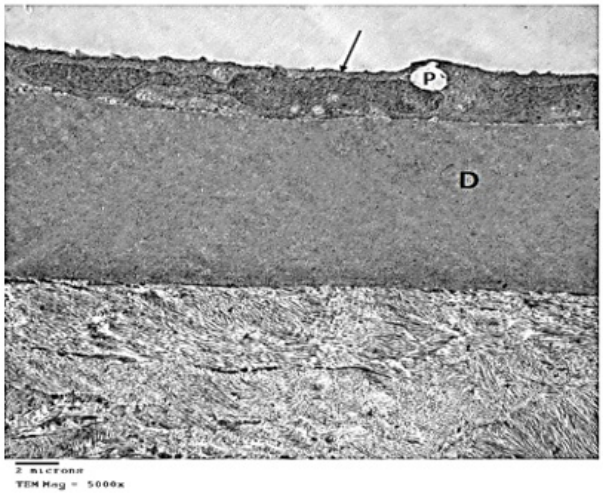

Figure 18: An electron micrograph of a section of cornea of Group III revealed homogenous thickened Descemet's membrane (D). Endothelial cells are of normal shape and size (↑) with pinocytic vesicle (P) in its cytoplasm TEM X5000.

Morphometric and statistical results

Morphometric and statistical results of subgroup Ib were non-significant (P>0.05), as compared to subgroup Ia. Concerning blood glucose level, it was significantly (P< 0.05) increased in Group II when compared with the control group. However, in Group III blood glucose level was significantly decreased (P<0.05) comparable with Group II with non-significant change when compared with Group I (Table 1).

	Group I	Group II	Group III
Blood glucose level mg/dl	105.2+21.3 (▲)	332.4+22.54 (*O)	107.2+26.2 (▲)
*Significant difference from Group I,▲ Significant difference from Group II, O Significant difference from Group III.			

Table 1: Mean ± SD of blood glucose level in different groups in mg/dl.

In diabetic group (Group II) there was a significant decrease (P<0.05) in the mean thickness of the corneal epithelium and total corneal thickness as compared with the other groups. Meanwhile, treatment with FDGL (Group III) revealed significant increase (P<0.05) in the mean thickness of corneal epithelium and total corneal thickness comparable to the diabetic group. In comparison to the control group, Group III revealed non-significant change in the mean thickness of corneal epithelium with significant increase (P<0.05) in the total corneal thickness (Table 2).

Corneal measurement	Group I	Group II	Group III
Mean of the thickness of corneal epithelium	40.3±1.8	16.4±2.1 (♦)	40.8±1.5 (*)
Mean of the total corneal thickness	186.7±12.3	106.2±6.3 (♦)	198.5±16.2 (▲*)
Significant decrease in comparison with Group I and Group III (♦), Significant increase in comparison with Group I (▲), Significant increase compared to Group II (*)			

Table 2: Showing mean ± SD of the corneal epithelium thickness and total corneal thickness in different groups in (µm).

Discussion

Long-term hyperglycemia has toxic effects on all cells in the body, and its most profound effects on eye tissues are on the cornea and retina. Seventy percent of diabetic patients are complicated by keratopathy, including recurrent corneal epithelial erosions, delayed wound healing, ulcers and edema [15]. Streptozotocin induced permanent diabetes mellitus in many animal species that resembles human diabetes patho-physiologically. Hence, STZ-induced diabetic rat is considered to be an animal model for developing new drugs for human diabetes mellitus [16]. Currently available oral antidiabetic drugs have certain drawbacks and therefore there is a need to find safer and more effective drugs. Accordingly, the current study was designed to investigate the ameliorative effect of FDGL on the structure of the cornea of STZ-induced diabetic rats. The corneal epithelium of the diabetic rats of the present study showed a significant decrease of its mean thickness comparable to that of the control group. This finding is in agreement with a previous study done by Daniel et al. The investigators attributed this to dry eye syndrome which is accompanied by an increase in apoptotic-driven surface cell desquamation. Subsequently, the epithelial surface cells loss is greater than the basal epithelial cells mitotic rate resulting in a net thinning of the corneal epithelium in the diabetic animals [17]. Moreover, other investigators postulated that, late stages of diabetes result in thinning of the corneal epithelium [18]. They attributed this to severe diabetic neuropathy which occurs in the late stages of diabetes as the corneal nerves may have a neurotrophic effect on the

corneal epithelial cells. The epithelial cells of the diabetic cornea of the present study showed darkly stained nuclei together with focal sloughing of the surface epithelial cells. Hemidesmosomal junctions appeared ill defined with interruption of the desmosomal junctions. Some investigators explained the abnormal epithelial morphology through some mechanisms which include the activation of the polyol pathway, accumulation of Advanced Glycation End Products (AGEs) and increased osmotic stress [19].

In the polyol pathway, the aldose reductase enzyme; which is presents in the cornea; metabolises excess glucose into sorbitol. Sorbitol is a sugar alcohol and strongly hydrophilic, and therefore it accumulates intracellularly with possible osmotic consequences and increased production of Reactive Oxygen Species (ROS) within the cell. Regarding AGEs, they arise from non-enzymatic reactions between extracellular proteins and glucose. AGEs alter the cell function by impairing the function of cellular proteins and lipids [19]. On the other hand, Shih et al**.** stated that hyperglycemia suppresses levels of epithelial growth factor receptor, ciliary neurotrophic factor and transforming growth factor beta-3 with consequential reduction in epithelial cell proliferation and increased apoptosis [20]. In the present study, corneal stroma in diabetic rats showed focal fragmentation and disruption of collagen fibrils. Some scientists explained this alteration in collagen biosynthesis and assembly in diabetes by accumulation of AGEs in the cornea stroma together with crosslinking between collagen molecules and proteoglycans [20]. Corneal stromal vascularization of the diabetic animals was detected in the present study. Corneal neovascularization in diabetes was recorded by some investigators who attributed that to sustained hyperglycemic condition with subsequent increase in the expression of Vascular Endothelial Growth Factor (VEGF) [21]. The whole corneal thickness of the diabetic rats in the present study showed a significant decrease of its mean thickness comparable to the control group. This finding was explained by Shih et al**.** who suggested that AGEs-related crosslinking of corneal proteins can change the shape

and morphology of the cornea in DM [20]. Another study was designed by Daniel et al. revealed significant decrease in central corneal thickness after 6 weeks of hyperglycemia in STZ induced diabetic mice [17]. They suggested that the widespread effects of hyperglycemia may impact and/or disrupt stromal development. They also mentioned the role of chronic hyperglycemic stress on keratocyte function and the downstream impact on stromal remodelling [17]. On the other hand, Rashmi and Bhawesh recorded statistically significant increase in central corneal thickness in diabetic patients than non-diabetics. They explained this increase by reduction of Na^+-K^+ ATPase activity which directly inhibits the corneal endothelial pump and accumulation of sorbitol, an osmotic agent causing corneal hydration [22].

In the current study, transmission electron microscopic examination of cornea of the diabetic group, revealed thickened Descemet's membrane with deposition of electron dense areas. The increased thickness and altered structure of Descemet's membrane were attributed by some researchers to the failure of protocollagen assembly, cross linking of collagen and or degeneration of endothelial cells [23]. In the present study, corneal endothelial cells of diabetic rats appeared distorted with irregular nuclei. Their cytoplasm revealed numerous large vacuoles as well as vacuolated mitochondria. These findings are in consistent with those of the previous study [24]. Moreover, El-Agmy's documented morphological anomalies of corneal endothelium in type 2 diabetes [25]. She demonstrated that hyperglycemia induce augmented activity of the aldose reductase enzyme leading to sorbitol accumulation in the corneal endothelial cells, which behaves as an osmotic agent producing corneal endothelium swelling in diabetic cornea. Moreover, Na^+-K^+ ATPase activity of the corneal endothelium in diabetic rats is decreased causing alterations of the corneal morphological and permeability features [25].

Nowadays, the medical world is turning more and more on the health benefits of natural products and medicinal herbs in the management of DM. Liquorice is one of the oldest herbs which are used for herbal treatment in Chinese medicine due to its wide pharmacological features [26]. However, one of the most commonly reported side effects of liquorice supplementation is elevated blood pressure, hypokalaemia and sodium retention [27]. This is thought to be due to pseudo-aldesterone-like effects of liquorice. The glycyrrhetenic acid; primary active component of liquorice; inhibits peripheral metabolism of cortisol, which binds to mineralocorticoid receptors in the same way as aldosterone [28]. Therefore, a Deglycyrrhizinated Liquorice (DGL) preparation was developed to provide some of the therapeutic benefits of liquorice while reducing its adverse effects [28].

Moreover, fermentation of liquorice produces large amounts of essential enzymes such as amylase and lipase which proved to be deficient in diabetic patients (both type 1,2) [8]. This fact inspired the role FDGL to replenish the stores of glycolytic enzymes as a replacement therapy. In the present study FDGL treatment of diabetic rats produced marked improvement in the histological structure of the cornea as the epithelial cells revealed intact hemidesmosomes and desmosomes. The construction of most of the substantia propria appeared almost similar to

that of the control group except for focal areas which revealed minimal separation of collagen fibrils. The mean thickness of all corneal layers revealed significant increase as compared to the diabetic group. Several mechanisms were suggested to mediate the anti-diabetic properties of DGL. Mehmet & Nevin attributed the hypoglycemic effect of DGL to chalcone and amorfrutin; which are active components of liquorice [26]. Licochalcone can increase the expression of peroxisome proliferator-activated receptor-γ (PPARγ) in white adipose tissues, which could enhance adipocyte differentiation and the population of small adipocytes and thereby improve hyperglycemia and hyperlipidemia associated with diabetes [29]. It is also hypothesized that glabridin, isoflavone in liquorice, potentiates glucose uptake through the Adenosine Monophosphate-Activated Protein Kinase (AMPK) pathway in skeletal muscle fibers. This pathway results in subsequent translocation of glucose transporter type 4 (GLUT4) to the cell membrane [30].

Furthermore, inhibition of α-glucosidase and α-amylase enzymes activity results in a decline in disaccharide hydrolysis which has beneficial effects on glycemic index control in diabetic patients and reduce the incidence of post prandial hyperglycemia. Karthikeson & Lakshmi assessed the *in vitro* anti-diabetic activity of liquorice. They reported the α-amylase inhibitory activity of Glycyrrhiza glabra ethanolic extract by 80.788 % [31]. Additionally, licochalcone A; active component of liquorice; has been shown to have inhibitory effects on protein tyrosine phosphatase1B (PTP1B), an enzyme with crucial role in the negative regulation of insulin and the leptin signalling pathway [32]. The antiapoptosis and anti-inflammatory activity of glycyrrhizic acid; active component of liquorice; against advanced glycation end product-induced damage in human umbilical vein endothelial cells has recently been demonstrated. It most probably occurs via inhibition of the receptor for advanced glycation end product/NF-κB pathway [33]. Besides its main active component glycyrrhizic acid, liquorice also consists of high amount of flavonoids, being responsible for most of the biological activities [5]. Fatemeh et al. elaborated that, antidiabetic properties of flavonoids are mediated through their effect on a number of molecular targets as well as regulation of several pathways [34]. These pathways include reducing apoptosis, improving proliferation of pancreatic β-cell and promoting insulin secretion; regulation of glucose metabolism in hepatocytes, decreasing insulin resistant and enhancing glucose uptake in skeletal muscle and adipose tissues. They also mentioned the promising effects of flavonoids in up-regulation of Glucose

Transporter Proteins (GLUT) expression levels which are integral membrane proteins that meditate transport of monosaccharides, polyols, and other small carbon compounds across the cell membranes [34]. Additionally, chronic hyperglycemia associated with diabetes enhances free radical production and decreases endogenous antioxidant defense, leading to tissue necrosis, inflammation, and fibrosis and organ damage [16]. One of the suggested mechanisms of flavonoids is their ability to protect and restore antioxidant defense enzymes such as superoxide dismutase, glutathione peroxidase and catalase, and inhibit ROS-producing enzymes such as xanthine oxidase [35]. As a consequence, flavonoids can prevent several ROS-stimulated biological events such as inhibiting oxidized LDL induced cell apoptosis, and NF-κB mediated transcriptional activity and subsequent inflammation [36] which found to be increased in the corneas of diabetic animals [20].

Conclusion and Recommendations

The results of the current study, proved that FDGL extract had considerable ameliorating effects on the diabetic keratopathy. However, there is still a wide diversity of questions remain open. Therefore, further studies are still highly desired for fully understanding of the new molecular targets for protection against DM.

Conflicts of interest

There are no conflicts of interest.

References

1. Vieira-Potter VJ, Dimitrios K, Darren J (2016) Ocular Complications of Diabetes and Therapeutic Approaches. BioMed Research International.
2. Nathan D (2015) Diabetes: advances in diagnosis and treatment. Journal of the American Medical Association 314: 1052-1062.
3. Jia Y, Jenny H, Cynthia C, Nan G, Feng W, et al. (2011) Corneal Complications in Streptozocin-Induced Type I Diabetic Rats. Investigative Ophthalmology & Visual Science 52: 6589-6596.
4. Ajit k, Pooja R (2017) Pharmacological Perspective of Glycyrrhiza glabra Linn: A Mini-Review. J Anal Pharma Res 5: 00156.
5. Giulia P, Laura C, Sónia S, Francisca R, Beatriz P (2018) Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytotherapy Research 32: 2323-2339.
6. Rizzato G, Scalabrin E, Radaelli M, Capodaglio G, Piccolo O (2017) A new exploration of licorice metabolome. Food Chemistry 221: 959-968.
7. Rajandeep K, Harpreet K, Ajaib D (2013) Glycyrrhiza Glabra: a phytopharmacological Review. IJPSR 4: 2470-2477.
8. Aughsteen A, Abu UM, Mahmoud S (2005) Biochemical analysis of serum pancreatic amylase and lipase enzymes in patients with type 1 and 2 diabetes mellitus. Saudi Med J 26: 73-77.
9. Massoud AMA (2011) European Patent No. EP 1925312B1. Retrieved from https://data.epo.org/gpi/EP1925312B1- ..
10. Buck W, Osweiter G, Van Gelder A (1976) Clinical and diagnostic veterinary toxicology. 2nd edn, Kendall, Hunt publishing Company, Iowa, p.52011.
11. Afifi N, Ramadan A, Abd El-Aziz M, Saki E (1991) Influence of dimethoate on testicular and epididymal organs, testosterone plasma level and their tissue residues in rats. Dtsch. Tierärzt. Wschr 98: 419-423.
12. Siham K, Hanan A, Ghada A (2014) Histological and immuno-histochemical study of beta cells in streptozotocin diabetic rats treated with caffeine. A histochemica et cytobiologica 52: 42-50.
13. Amaral S, Santos M, Seica R, Ramalho J (2006) Effects of hyperglycemia on sperm and testicular cells of Gotom-Kakizaki and streptozotocin -treated rat model for diabetes. Theriogenology 66: 2056-2067.
14. Bancroft JD, Layton C, Suvarna SK (2013) Bancroft's Theory and Practice of Histological Techniques. 7th edn. Elsevier Churchill Livingstone: London, United Kingdom 386-535.
15. Feng G, Tao L, Yingzhe P (2016) Effects of diabetic keratopathy on corneal optical density, central corneal thickness, and corneal endothelial cell counts. Experimental and Therapeutic Medicine 12: 1705-1710.
16. Amal A, Chinnadurai V, Chandramohan G, Mohammed A, Ahmed S, et al. (2017) Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. REDOX REPORT 22: 290-300.
17. Daniel C, Meifang Z, Matthew P, Vindhya K, Danielle M (2014) The Impact of Type 1 Diabetes Mellitus on Corneal Epithelial Nerve Morphology and the Corneal Epithelium. The American Journal of Pathology 184: 2662-2670.
18. Rosenberg M, Tervo T, Immonen I, Muller L, Gronhagen-Riska C, et al. (2000) Corneal structure and sensitivity in type 1 diabetes mellitus. Invest Ophthalmol Vis Sci 41: 2915-2921.
19. Solani D, Tshegofatso M (2015) Is the central corneal thickness of diabetic patients thicker than that of non-diabetics' eyes? Afr Vision Eye Health 74: 1.
20. Shih K, Lam K, Tong L (2017) A systematic review on the impact of diabetes mellitus on the ocular surface. Nutrition & Diabetes 7: e251.
21. Tomohiko S (2010) Pathophysiological Characteristics of Diabetic Ocular Complications in Spontaneously Diabetic Torii Rat. Journal of Ophthalmology.
22. Rashmi K, Bhawesh C (2017) Central Corneal Thickness and Diabetes - A Study of Correlation in Terms of Duration and Glycemic Control. International Journal of Contemporary Medical Research 4: 767-769.
23. Take G, Karabay G, Erdogan D, Duyar I (2006) The ultrastructural alterations in rat corneas with experimentally-induced diabetes mellitus. Saudi Med J 27: 1650-1655.
24. Rajni S, Aakash P, Harita S, Roshani P, Toral R (2018) A study of correlation between HbA1c level & corneal thickness in diabetes mellitus patients. Indian Journal of Clinical and Experimental Ophthalmology 4: 96-99.
25. El-Agamy A (2017) Corneal Endothelium and Central Corneal Thickness Changes in Type 2 Diabetes Mellitus. Insights in Ophthalmology 1: 2.

26. Mehmet A, Nevin SA (2017) Review: Pharmacological Effects of Licorice (Glycyrrhiza glabra) on Human Health. IJBCS 6: 12-26.

27. Jalal Z, Zahra K, Masoud G (2013) Licorice (Glycyrrhiza glabra Linn) As a Valuable Medicinal Plant. Int J Adv Biol Biom Res 1: 1281-1288.

28. Ghulam D & Muhammad R (2016) Glycyrrhiza glabra L. (Liquorice). Pak. J. Pharm. Sci 29: 1727-1733.

29. Park H, Bak E, Woo G, Kim JM, Quan Z, et al. (2012) Licochalcone E has an an-tidiabetic effect. J Nutr Biochem 23: 759-767.

30. Sawada K, Yamashita Y, Zhang T, Nakagawa K, Ashida H (2014) Glabridin induces glucose uptake via the AMP-activated protein kinase pathway in muscle cells. Molecular and cellular endocrinology 393: 99-108.

31. Karthikeson P, Lakshmi T (2017) Anti-Diabetic Activity of Glycyrrhiza glabra - An *in vitro* Study. Int. J. Pharm. Sci. Rev. Res 44: 80-81.

32. Hossein H, Marjan N (2015) Pharmacological Effects of Glycyrrhiza spp. and Its Bioactive Constituents: Update and Review. Phytother. Res 29: 1868-1886.

33. Feng L, Zhu M, Zhang M, Wang RS, Tan XB, et al. (2013) Protection of glycyrrhizic acid against AGEs-induced endothelial dysfunction through inhibiting RAGE/NF-κB pathway activation in human umbilical vein endothelial cells. J Ethnopharmacol 148: 27-36.

34. Fatemeh H, Manizheh K, Aditya A (2015) Modulation of Glucose Transporter Protein by Dietary Flavonoids in Type 2 Diabetes Mellitus. Int. J. Biol. Sci 11: 508-524.

35. Hana A, Yao W, Dongmin L (2018) Flavonoids in the Prevention of T2D: An Overview. Nutrients 10: 438

36. Yi L, Chen C, Jin X, Zhang T, Zhou Y, et al. (2012) Differential suppression of intracellular reactive oxygen species-mediated signaling pathway in vascular endothelial cells by several subclasses of flavonoids. Biochimie 94: 2035-2044.