



## Virgin Olive Oil Protects the Cornea against Diabetes-Induced Damage in Rats: A Biochemical and Histological Study

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## General Note

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## ABSTRACT

Diabetes mellitus (DM) is a chronic health problem with a growing spread universally. Diabetes poses severe health complications, including neuropathy, cardiomyopathy, and ocular difficulty which is a typical complaint between diabetic subjects. Virgin olive oil is a natural product rich in phenols and other antioxidants. It is commonly recognized to protect against numerous ailments and disorders, including diabetes. The main objective of the present study was to investigate the possible protective role of virgin olive oil against diabetic induced corneal histopathological changes in streptozotocin (STZ) induced diabetic rats. Furthermore, the underlined mechanism was investigated regarding the antioxidant's capacity. Diabetes was induced by giving a single intraperitoneal (i.p.) injection of STZ (40 mg/kg bw). Twenty-four male Sprague-Dawley rats were allocated in 4 equal groups: non-diabetic control, STZ- diabetic, diabetic and olive oil (1.0 ml/100 gbw/day), and diabetic and metformin (500 mg /kg/bw/day). The experiment design for treatment last for six weeks. Pathological examination of the corneal tissue was used to ascertain the potential protective effect of virgin olive oil against the damage associated with diabetes. The results of this study showed a glycemic and glycosylated hemoglobin lowering effect of virgin olive oil and metformin in STZ-induced diabetes. Moreover, olive oil and metformin reduced the lipid peroxidation product and increased the level of total antioxidants capacity. Furthermore, this study showed an ameliorative effect of olive oil on diabetic induced corneal histopathological complications. In conclusion, olive oil seemed to protect against diabetic induced changes in rat's cornea as it maintained epithelium integrity and prevented keratinization and stroma neovascularization via both controlling blood glucose level and most probably via antioxidant activity.

**Keywords:** Diabetes, olive oil, metformin, HbA1c, glucose, antioxidant, cornea, histopathology

## 1. INTRODUCTION

Diabetes mellitus (DM) is a severe health problem with a growing spread universally. It is approximated that the figure of diabetes in adults will attain 642 million by the year 2040 (Lau et al., 2019). Diabetes posed severe health complications, including most common neuropathy (Himeno et al., 2020; Zochodne, 2019) and cardiac disorders (Murtaza et al., 2019; Richter et al., 2020). The ocular complication is a typical sequence among diabetic patients (Gudla et al., 2018; Craig et al., 2014). Diabetic keratopathy has been estimated to occur in 47%–64% of diabetic patients during their disease (Priyadarsini et al., 2020). Unlike diabetic retinopathy or cataracts, diabetic keratopathy patients usually do not have detectable symptoms; however, once the cornea is injured, delayed epithelial wound healing is often observed (Chikama et al., 2007) and may be accompanied by vision-minatory problems like microbial keratitis, stromal opacification, and surface inconsistency (Pflugfelder, 2006).

The human cornea is an avascular transparent structure, has five layers. The surface epithelium of the cornea is an extremely thin multicellular layer characterized by the ability of rapid growth and easily regenerated. The epithelium is resting on a homogenous membrane, which is called the Bowman membrane and is critically important for maintaining epithelium integrity. The third layer is known as Substantia propria and consisting of regularly arranged collagen fibers with interconnected keratocytes. Descemet membrane bordering substantial propria and separating it from the most inner corneal endothelium (Sridhar, 2018).

Free radicals' generation, oxidative stress, and inflammatory processes evoked by hyperglycemia are beyond changes occurred in corneal layers leading to derangement of its transparency, normal refraction that compromised visual acuity (Yaribeygi et al., 2019).

Virgin olive oil is a natural product rich in phenols and other antioxidants. It is commonly recognized to protect against numerous ailments and disorders (Reboredo-Rodríguez et al., 2018). The phenolic active constituents of olive oil possess antioxidant and anti-inflammatory activities. Besides, they own positive influence on the diverse physiological processes, like antibacterial activity, bone parameters, inflammatory indicators, oxidative injury, and plasma lipoproteins (Cicerale et al., 2010; Cicerale et al., 2012). Diet wealthy in olive oil is additionally familiar to alleviate diabetes. Furthermore, Olive oil showed an ameliorative effect on hyperglycemic animals that can be helpful to keep normal blood glucose and prohibit the start of hyperglycemic complications (Balamash et al., 2018; Schwingshackl et al., 2017).

Based on previous data, the main objective of the present study was to investigate the possible protective role of virgin olive oil against diabetic induced corneal histopathological changes in streptozotocin (STZ) induced diabetic rats.

## 2. MATERIALS AND METHODS

### **Materials**

Glucophage of Merck, Germany (metformin 1000 mg); STZ of Sigma-Aldrich, USA, and virgin olive oil of Aladhara Agricultural Project, Saudi Arabia were utilized in this research.

### **Animals**

Twenty-four adult male Sprague-Dawley rats with average body weight 200-250 g were obtained from animal house unit in King Fahad Medical Research Center in King Abdul-Aziz University, Jeddah, Saudi Arabia. Acclimatization to lab condition (kept at room temperature and maintain the 12 h light and dark period) was done for one week and fed with the standard rat pellets. The experiment started from on May 2018 and ended on October 2018.

### **Ethical approval**

The study's plan was reviewed via the Biomedical Ethics Committee, College of Medicine, University of King Abdul-Aziz, Saudi Arabia (Experimental Study, Reference No 142-16).

### **Methods**

Diabetes was induced by giving a single intraperitoneal (i.p.) injection of STZ (40 mg/kg) dissolved in 0.05M citrate buffer (pH 4.5), (Motyl and McCabe, 2009). Glucose solution 10% was given for 24 h to guard against hypoglycemia. The concentration of blood glucose was assessed after 72 hours where the concentration of 250 mg/dl was an indicator for chosen diabetic rats to be included in the experiment (King, 2012). The following groups were planned for the research: Group 1: nondiabetic control; Group 2:STZ-diabetic; Group 3: diabetic and olive oil (virgin olive oil was given orally at a dose 1.0 ml/100 gmbw/day) all through the experiment; Group 4: diabetic and metformin (metformin was given orally at a dose 300 mg /kg daily for 2 weeks then 500 mg for the rest of 4 weeks), (Balamash et al., 2018). The experiment design for treatment last for six weeks.

### **Blood and cornea collection**

At the termination of the six weeks, rats were ether anesthetized, and blood specimens were withdrawn from the retro-orbital plexus to isolate the serum. Serum was obtained by centrifuging the coagulated blood samples for 15 min at 4000 rpm. The isolated serum was preserved frozen at -80 °C till used for the biochemical assays. The cornea from all groups of rats was excised and fixed with buffered formalin (10%).

### **Assessment of serum glucose, glycated hemoglobin (HbA1c), and insulin concentration**

The Glucose Flex® Reagent Cartridge (Siemens Healthcare Diagnostic Inc, Newark, USA) was used to measure fasting serum glucose concentration of all groups.

The ELISA assay kit rat specific (My BioSource, San Diego, California, USA) was used to measure serum HbA1c concentration of all groups.

The ELISA assay kit rat specific (Merck Millipore, Billerica, USA) was used to measure serum fasting insulin concentration of all groups.

### **Assessment of serum malondialdehyde (MDA) and total antioxidant capacity (TAO) concentration**

The OxiSelect™ assay kit (Cell Biolabs, Inc, San Diego, USA) was used to measure serum TAO concentration of all groups.

The UV colorimetric assay kit (Cayman Chemical, Ann Arbor, Michigan, USA) was used to measure serum MDA concentration of all groups.

### **Histopathological examination**

Paraffin sections of the formalin-fixed cornea were sectioned as 3-5 µm slices for hematoxylin and eosin (H & E) staining. The slides were photographed (Olympus DP 72 camera) under the light microscopy (Olympus BX51TF).

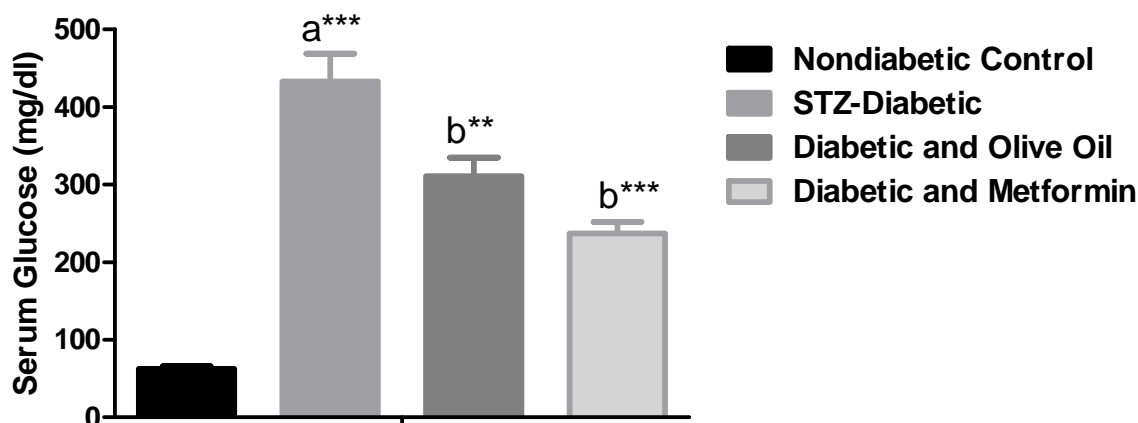
### **Statistical calculations**

The findings of this study were statistically studied utilizing GraphPad Prism Statistical Software version 5.0. The results were offered as mean ± standard error (SE). Significance between treatments was assessed utilizing the one-way analysis of variance (ANOVA). A p-value < 0.05 was significant.

### 3. RESULTS

#### **Impact of virgin olive oil on serum glucose concentration**

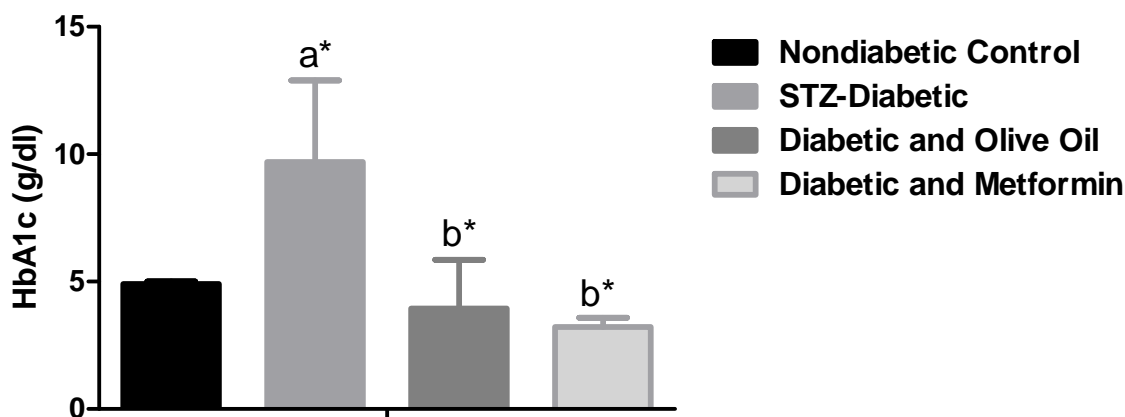
The group of rats induced with diabetes displayed a significant increase in the concentration of serum glucose contrast to the control group ( $p < 0.001$ ). The therapy of diabetic rats with both virgin olive oil and metformin lowered the serum glucose concentration in a statistically significant way ( $p < 0.01$  and  $p < 0.001$ , respectively) contrast to the diabetic group. No statistical variation was observed among the olive oil treatment group and the metformin treatment group. The glucose concentration in the two treatment groups did not return to its normal concentration in the control group (Figure 1).



**Figure 1.** Impact of virgin olive oil and metformin on fasting serum glucose concentration measured in STZ-prompted diabetic rats. Results are presented as arithmetic average  $\pm$  SE ( $n = 6$ ).<sup>a\*\*\*</sup> Significant contrast to nondiabetic control ( $p < 0.001$ ); <sup>b\*\*\*</sup> Significant contrast to STZ-diabetic ( $p < 0.001$ ); <sup>b\*\*</sup> Significant contrast to STZ-diabetic ( $p < 0.01$ ).

#### **Impact of virgin olive oil on serum glycated hemoglobin (HbA1c) concentration**

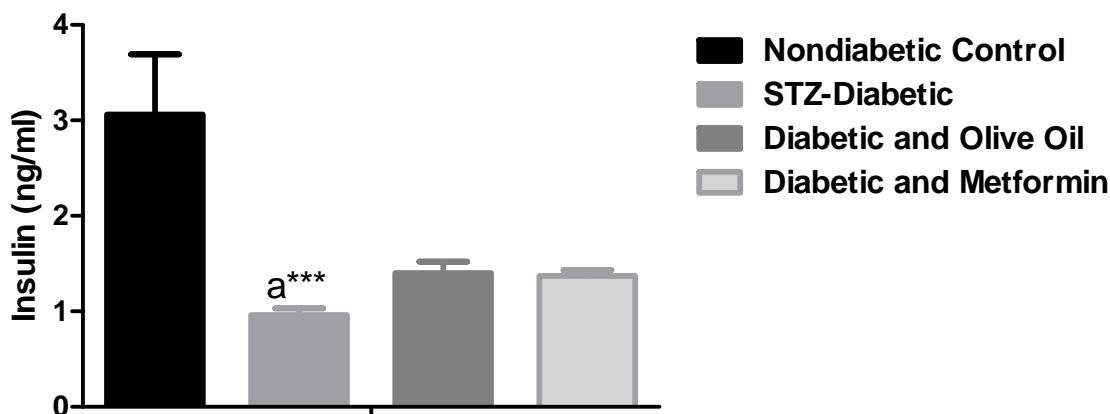
The group of rats induced with diabetes displayed a significant increase ( $p < 0.05$ ) in the concentration of serum HbA1c contrast to the control group. The therapy of diabetic rats with both virgin olive oil and metformin lowered the serum HbA1c concentration in a statistically significant way ( $p < 0.05$ ) contrast to the diabetic group. No statistical variation was observed among the olive oil treatment group and the metformin treatment group. The HbA1c concentration in the two treatment groups returned to its normal concentration in the control group (Figure 2).



**Figure 2.** Impact of virgin olive oil and metformin on serum glycated hemoglobin (HbA1c) concentration measured in STZ-prompted diabetic rats. Results are presented as arithmetic average  $\pm$  SE ( $n = 6$ ).<sup>a\*</sup> Significant contrast to nondiabetic control ( $p < 0.05$ ); <sup>b\*</sup> Significant contrast to STZ-diabetic ( $p < 0.05$ ).

### Impact of virgin olive oil on serum insulin concentration

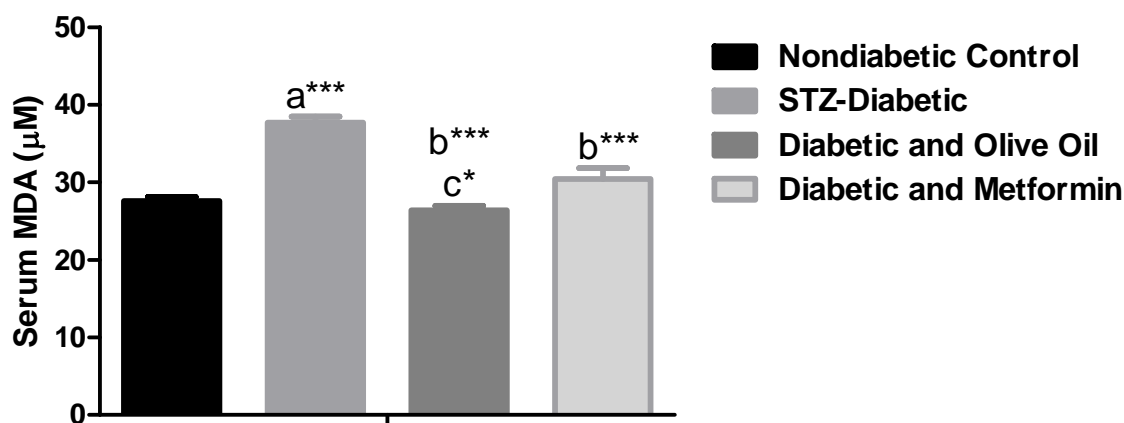
The group of rats induced with diabetes displayed a significant decrease ( $p < 0.001$ ) in the concentration of serum insulin contrast to the control group. The therapy of diabetic rats with both virgin olive oil and metformin exerted no effects on the serum insulin concentration contrast to the diabetic group. No statistical variation was observed among the olive oil treatment group and the metformin treatment group. The insulin concentration in the two treatment groups did not return to its normal concentration in the control group (Figure 3).



**Figure 3.** Impact of virgin olive oil and metformin on fasting serum insulin concentration measured in STZ-prompted diabetic rats. Results are presented as arithmetic average  $\pm$  SE ( $n = 6$ ). a\*\*\* Significant contrast to nondiabetic control ( $p < 0.001$ ).

### Impact of virgin olive oil on serum malondialdehyde (MDA) concentration

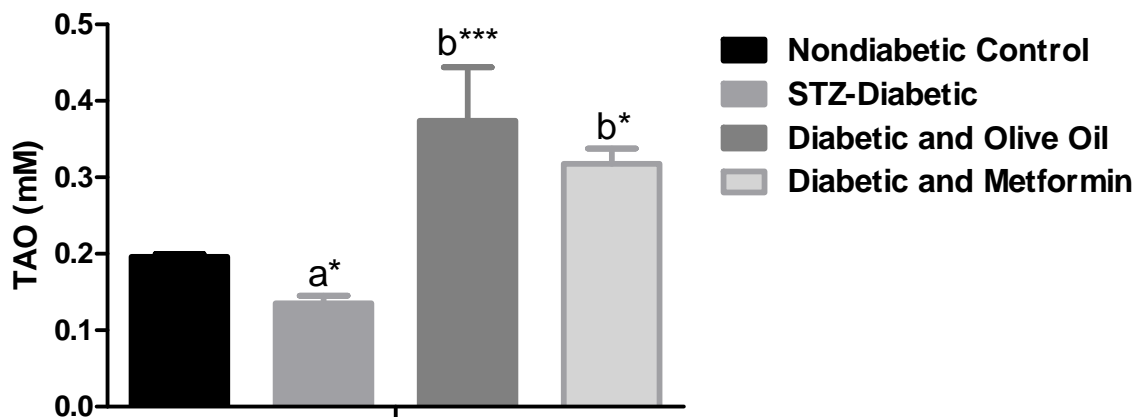
The group of rats induced with diabetes displayed a significant increase in the concentration of serum MDA contrast to the control group ( $p < 0.001$ ). The therapy of diabetic rats with both virgin olive oil and metformin lowered the serum MDA concentration in a statistically significant way ( $p < 0.001$ ) contrast to the diabetic group. A statistical variation was calculated among the olive oil treatment group and the metformin treatment group ( $p < 0.05$ ). The MDA concentration in the two treatment groups returned to its normal concentration in the control group (Figure 4).



**Figure 4.** Impact of virgin olive oil and metformin on serum malondialdehyde (MDA) concentration measured in STZ-prompted diabetic rats. Results are presented as arithmetic average  $\pm$  SE ( $n = 6$ ). a\*\*\* Significant contrast to nondiabetic control ( $p < 0.001$ ); b\*\*\* Significant contrast to STZ-diabetic ( $p < 0.001$ ); c\* Significant contrast to diabetic and metformin ( $p < 0.05$ ).

### Impact of virgin olive oil on serum total antioxidant capacity (TAO) concentration

The group of rats induced with diabetes displayed a statistically significant decrease ( $p < 0.05$ ) in the concentration of serum TAO contrast to the control group. The therapy of diabetic rats with both virgin olive oil and metformin elevated the serum TAO concentration in a statistically significant way ( $p < 0.001$  and  $p < 0.05$ , respectively) contrast to the diabetic group. No statistical variation was observed among the olive oil treatment group and the metformin treatment group. The TAO concentration in the two treatment groups exceeded the normal concentration in the control group (Figure 5).



**Figure 5.** Impact of virgin olive oil and metformin on serum total antioxidant (TAO) concentration measured in STZ-prompted diabetic rats. Results are presented as arithmetic average  $\pm$  SE ( $n = 6$ ).<sup>a\*</sup> Significant contrast to nondiabetic control ( $p < 0.001$ );<sup>b\*\*\*</sup> Significant contrast to STZ-diabetic ( $p < 0.001$ ); <sup>b\*</sup> Significant contrast to diabetic and metformin ( $p < 0.05$ ).

### Impact of virgin olive oil and metformin on corneal tissue histopathology

The cornea of nondiabetic control rats showed intact epithelium, basement membrane, and endothelium, besides homogenous regular collagen and corneoblasts. The cornea of the diabetic rats showed thinning of epithelial layers, epithelial spongiosis, and superficial keratinization. The underlying lamina showed an edematous separation of collagen. Decreased corneoblasts and signs of vascularization and inflammatory cell infiltrate. The endothelial layer showed disrupted epithelium. The treatment of diabetic rats with both virgin olive oil and metformin markedly preserve of normal corneal structure (Figure 6).

## 4. DISCUSSION

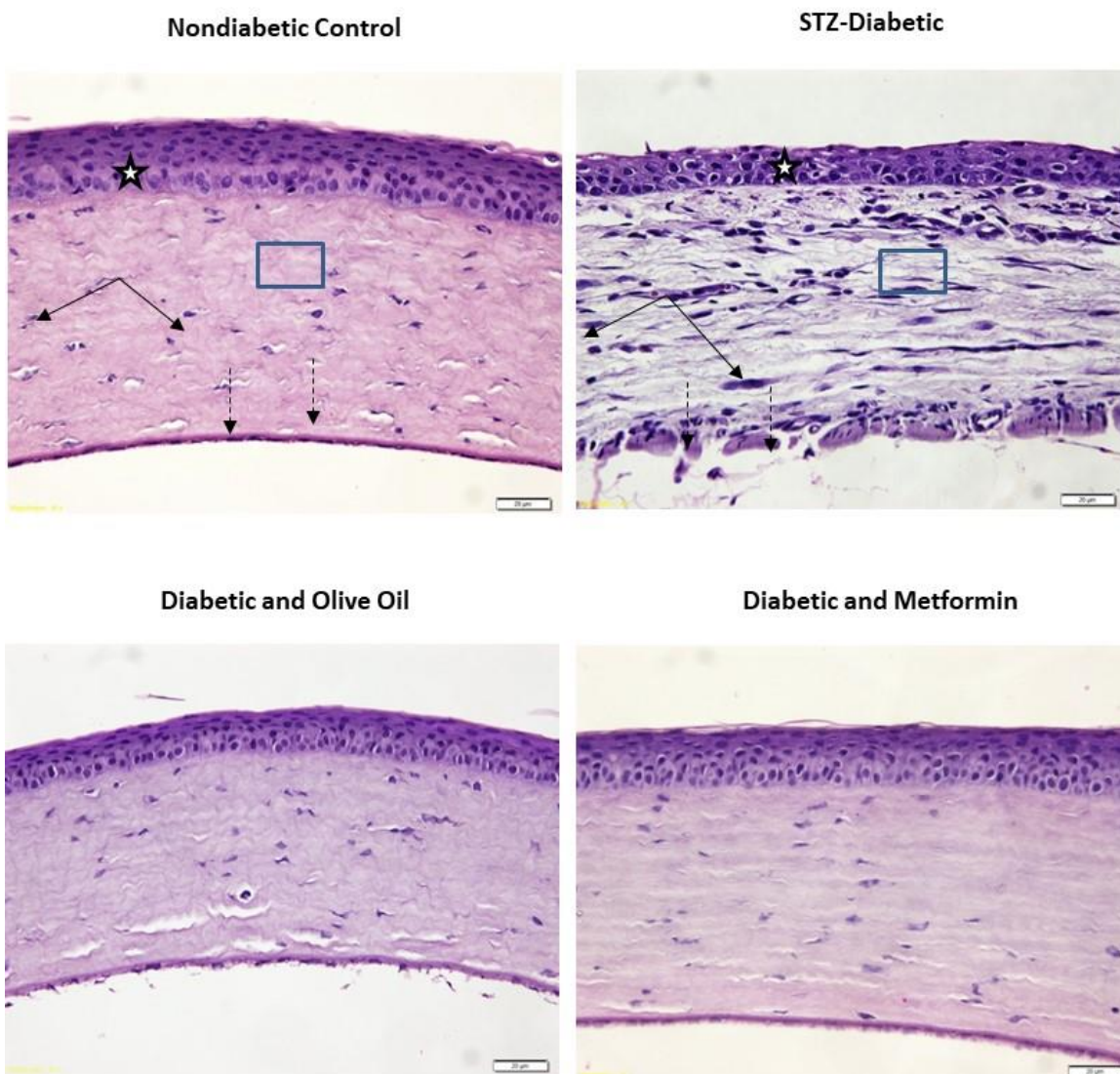
The defensive impact of virgin olive oil was examined in the present research through studying the pathological alterations of the cornea in STZ-induced diabetic rats. This research showed the improvement impact of virgin olive oil on STZ-prompted diabetes in rats that may be helpful to prohibit diabetes- prompted corneal problems. The results of this study also showed a glycemic and glycosylated hemoglobin lowering effect of virgin olive oil in STZ-produced diabetes. Moreover, olive oil reduced the lipid peroxidation product and increased the level of total antioxidants capacity. In agreement with the results of this study, researchers recently reported that olive oil supplements could reduce both fasting blood sugar and glycosylated hemoglobin in type 2 diabetics (Schwingshackl et al., 2017).

Several pathophysiological abnormalities have been noted in diabetic keratopathy, including, an abnormally thickened and discontinuous basement membrane, abnormal adhesion between the stroma and basement membrane (Jeng et al., 1991; Simpson et al., 2012; Gipson et al., 1983; Gipson et al., 1987; McDermott et al., 2003), increased epithelial fragility (Yang et al., 2006), decreased epithelial healing rates, increased sorbitol concentrations (Yue et al., 1982), decreased oxygen consumption and uptake (Graham et al., 1981), increase in the polyol metabolism (Kinoshita et al., 1979), decreased or altered epithelial hemidesmosomes, and increased glycosyl transferase activity (Lu and Watsky, 2019).

Delayed epithelial injury recovery, edema, frequent ulceration, neuropathy/loss of sensitivity, and tear film alteration are recurrent but unidentified complications of both diabetes mellitus types; (insulin-dependent, type 1) and (non-insulin-dependent, type 2), (Ljubimov, 2017). Those diabetic complications evaluated to be inspired by two leading pathogenic processes, permanent oxidative stress raised by free radical liberation and persistent low-grade inflammation (Ljubimov, 2017). Increased blood glucose levels can disrupt the natural antioxidant defenses. Generally, oxygen-derived free radicals are quickly removed by antioxidants.



Research offered that plasma and tissue concentration of the antioxidant, vitamin C was 40 to 50% reduced in diabetic subjects contrast with nondiabetic subjects (Aronson and Rayfield, 2002).



**Figure 6.** Effect of virgin olive oil and metformin on corneal tissue histopathology determined in STZ-induced diabetic rats. **Photo of nondiabetic control** rat showed an intact epithelium and basement membrane (star). The underlying substantia propria showed homogenous regular collagen and corneoblasts (square and arrows). The last layer showed healthy, intact endothelium (dotted arrows). **Photo of STZ-diabetic** rat showed thinning of epithelial layers, epithelial spongiosis, and superficial keratinization. The underlying lamina showed an edematous separation of collagen. Decreased corneoblasts and signs of vascularization and inflammatory cell infiltrate. **Photo of diabetic and olive oil** rat showed marked preservation of the normal corneal structure. **Photo of diabetic and metformin** rat showed marked preservation of the normal corneal structure.

It is possible that the mechanism behind the protective effect of olive oil against the corneal damage associated with diabetes depends on the antioxidant mechanism of olive oil. Olive oil holds a huge amount of monounsaturated fatty acids mainly oleic acid (70-80%) besides saturated fatty acids mainly palmitic acid (10-15%) and omega 3, 6, and 9 polyunsaturated fatty acids (5-10%). Olive oil was reported exerting hypoglycemic, antioxidant, and anti-inflammatory characteristics (Parrilha et al., 2015; Waterman and Lockwood, 2007). In addition to this, the protective effect of olive oil against the pathological changes in the cornea may lie in its control of the level of blood sugar and glycated hemoglobin.

## 5. CONCLUSION

Olive oil seemed to provide protection against diabetic induced changes in rat cornea as it maintained epithelium integrity and prevented keratinization and stroma neovascularization via both controlling blood glucose level and most probably via antioxidant activity. The latter needs future confirmation through studying corneal antioxidant contents.

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This study has not received any external funding.

### Conflict of interest

The authors declare that there are no conflicts of interest.

### Data and materials availability

All data associated with this study are present in the paper. The data supporting the result of this research are consistent with the previous author on appropriate request.

### Peer-review

External peer-review was done through double-blind method.

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