Therapeutic Effects of Sunitinib on Diabetes Mellitus Related Ovarian Injury: An Experimental Rat Model Study

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High levels of glucose causes tissue injury through 5 major mechanisms:

- polyol pathway
- activation of protein kinase-C isoforms
- overactivity of hexosamine pathway
- ↑ intracellular advanced glycation end products (AGEs)
- ↑ expression of the receptor for AGEs and its activating ligands

Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625.

Intracellular AGE precursors' production can damage cells by 3 ways;

- intracellular proteins modified by AGEs have altered function
- extracellular matrix compounds modified by AGE precursors interact abnormally with other matrix components
- plasma proteins modified by AGE precursors bind to AGE receptors (RAGE) on cells

Stitt AW, Moore JE, Sharkey JA, Murphy G, Simpson DA, Bucala R, Vlassara H, Archer DB. Advanced glycation end products in vitreous: structural and functional implications for diabetic vitreopathy. Invest Ophthalmol Vis Sci 1998;39:2517–2523.

Stitt AW, Li YM, Gardiner TA, Bucala R, Archer DB, Vlassara H. Advanced glycation end products (AGEs) co-localize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am J Pathol 1997;150:523–531.

- RAGE → production of ROS → pleiotropic transcription factor nuclear factor (NF)-kappaB → multiple pathological changes in gene expression

- Activation of NF-kappaB pathway → production of Vascular Endothelial Growth Factor (VEGF) and inflammatory cytokines like TNF and IL-1

- Like the other organ systems, glucose toxicity occurs severely in ovary and this injury is originated by NF-kappaB way in DM

Kiriakidis S, Andreakos E, Monaco C, Foxwell B, Feldmann M, Paleolog E. VEGF expression in human macrophages is NF-kappaB-dependent: studies using adenoviruses expressing the endogenous NF-kappaB inhibitor IkappaBalpha and a kinase-defective form of the IkappaB kinase 2. J Cell Sci 2003;116:665-674.
Pala HG, Erbas O, Oltulu F, Pala EE, Aktug H, Yavasoglu A. Glucose Injury in Diabetic Rat's Ovaries and Effect of NF-Kappa B Way. Ege J Med 2013;52:32-36.

- Sunitinib → oral, tyrosine kinase inhibitor FDA → gastrointestinal stromal tumors and advanced stage renal cell carcinoma

- Investigated in adhesion prevention
- More specific for VEGF receptors
- Activity against platelet derived growth factor, stem cell factor receptor (C-kit), glial cell-line derived neutrophilic factor receptor (RET) and Fms-like tyrosine kinase-3

Patel TV, Morgan JA, Demetri GD, et al. A preeclampsia-like syndrome characterized by reversible hypertension and proteinuria induced by the multitargeted kinase inhibitors sunitinib and sorafenib. J Natl Cancer Inst 2008;100:282-284.
Launay-Vacher V, Derey G. Hypertension and proteinuria: a class-effect of antiangiogenic therapies. Anticancer Drugs 2009;20:81-82.

Aim of the study

Investigate the effects of sunitinib on diabetes mellitus related-ovarian injury and fibrosis in rat models

Materials & Methods

- 24 female Sprague Dawley albino mature rats at 8 weeks, weighing 200–220 gram
- fed ad libitum
- 22 ± 2 °C
- 12-hour light/dark cycles
- Ethic Committee for Animal Research of Gaziosmanpasa University
- Animal experiment guidelines of the Committee for Human Care

Experimental protocol

- Diabetes → i.p. injection of STZ *(Sigma-Aldrich, Inc.; Saint Louis, MO, USA)* (60 mg/kg in 0.9% NaCl, adjusted to a pH 4.0 with 0.2M sodium citrate) for 16 rats
- No drug → remainder of rats [blood glucose levels were ↓ 120 mg/dl (n=8) (control group, Group-1)

Experimental protocol

- Diabetes was verified after 24 hours by evaluating blood glucose levels with the use of glucose oxidase reagent strips (Boehringer- Mannheim, Indianapolis).

- The rats with blood glucose levels 250 mg/dl ↑ → diabetic rat group (n=16)

Experimental protocol

- STZ → 7 weeks → development of diabetes-related microvascular complications
- 16 diabetic rats → randomly divided into 2 groups;
- Group-2 (diabetic control group, 8 rats) → no medication (4 ml/day tap water by oral gavage)
- Group 3 (sunitinib group, 8 rats) →
  1 mg/kg/day oral sunitinib for 4 weeks

Histopathological examination

- Rats euthanized → bilateral oophorectomy
- Formalin-fixed ovary sections (4 µm) were stained with hematoxylen & eosine

- Follicular degeneration
- Stromal degeneration
- Stromal fibrosis

  scored from 0 to 3 according to the injury severity

- 0 → no pathologic findings
- 1 → 33% ↓
- 2 → 33% - 66%
- 3 → 66% ↑

NF-kappaB immunoexpression

- For immunohistochemistry, sections → H_2O_2 (10%). → with primary antibodies *(NF-kappaB, Bioss Inc.; dilution 1/100)*
- Antibody detection → Histostain-Plus Bulk kit *(Bioss Inc.)* against rabbit IgG, and 3,3' diaminobenzidine (DAB) was used to visualise the final product
- The number of NF-kappaB positive cells was assessed systematically by scoring at least 100 ovarian stromal cells per 10 fields of tissue sections at 100x

Statistical analysis

- SPSS version 20.0 for Windows
- Parametric → Student's t test and ANOVA
- Nonparametric → Mann Whitney U test
- Cathegorical variables → x^2 test
- Mean ± standard error of mean (SEM)
- $p < 0.05$ → statistically significant
- $p < 0.001$ → statistically highly significant

Results

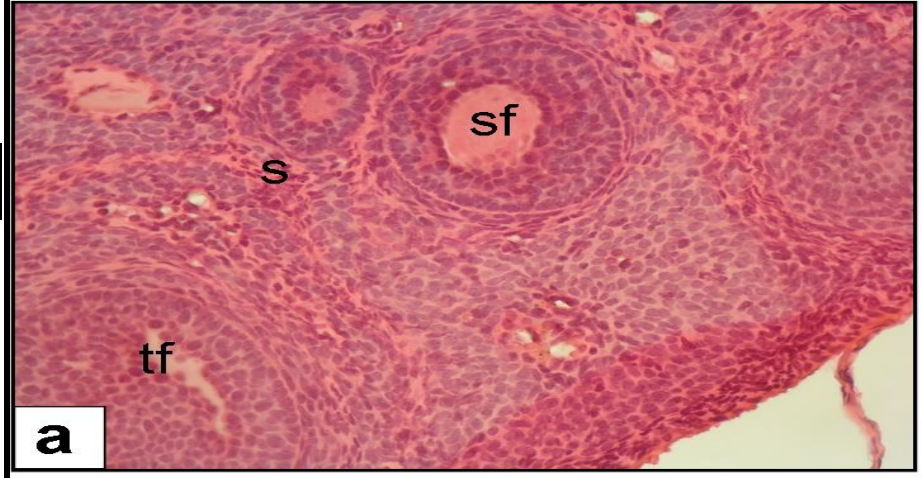
- Follicular degeneration, stromal degeneration, stromal fibrosis and NF-kappa B immune-expression were statistically significantly higher in non-treated diabetic rat's ovary (Group-2) when compared with control group rats (Group-1) $\rightarrow$ ($p<0,0001$)

Results

- Stromal degeneration (p=0,04)
- Stromal fibrosis (p=0,01)
- Follicular degeneration (p=0,02)
- NF-kappa B immune-expression (p=0,001) → statistically significantly lower in sunitinib-treated diabetic rat's ovary (Group-3) when compared with non-treated diabetic rat's ovary (Group-2)

	Stromal Degeneration Score	Follicle Degeneration Score	Stromal Fibrosis Score	NF-kappaB Immunoexpression percent (%)
Normal Control (Group-1)	0,18 ± 0,03	0,21 ± 0,10	0,28 ± 0,07	4,33 ± 1,15
Diabetic rat (non-treated) (Group-2)	2,24 ± 0,21 *	2,65 ± 0,35 *	2,58 ± 0,54 *	38,14 ± 3,38 *
Sunitinib (Group-3)	1,28 ± 0.18 **	1,43 ± 0.32 **	1,16 ± 0.15 **	11.23 ± 5.45 **

* p<0,0001, Normal control group (Group-1) compared with diabetic rat (non-treated) group (Group-2)
** p<0,05, Sunitinib group (Group-3) compared with diabetic rat (non-treated) group (Group-2)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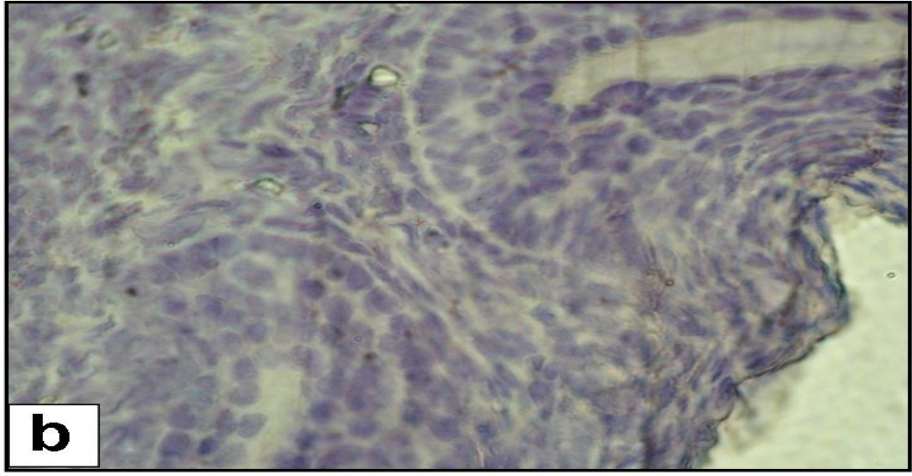
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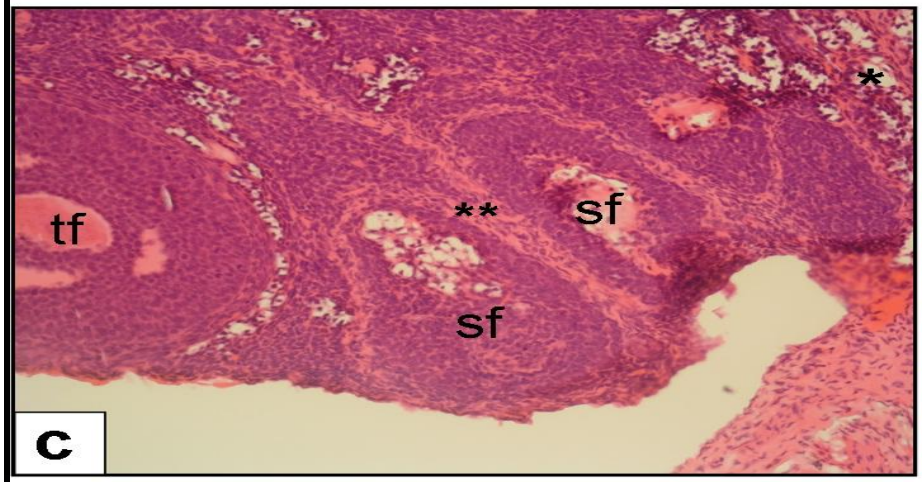
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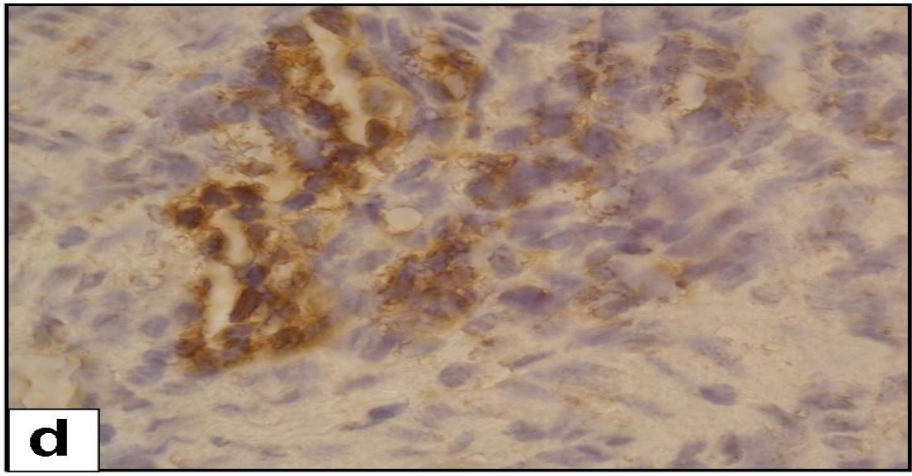
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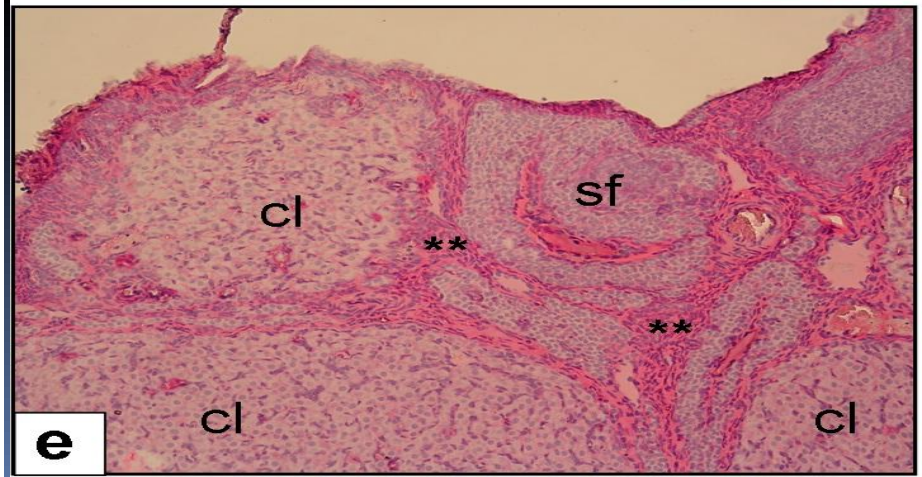
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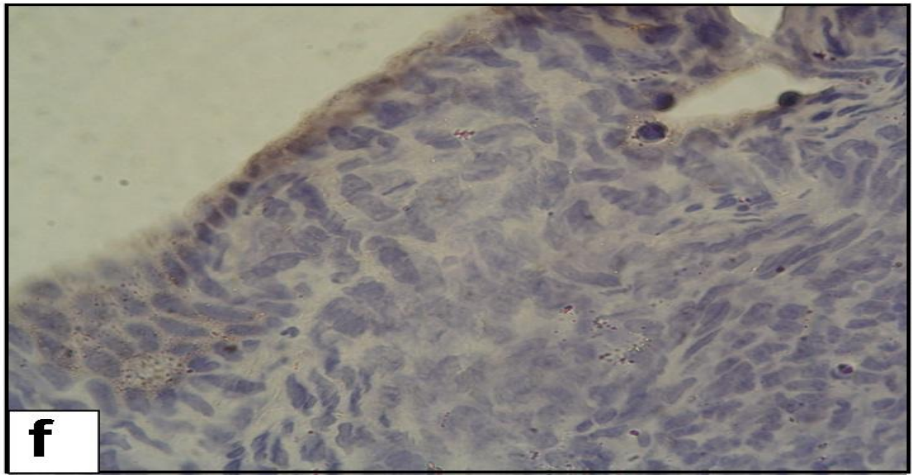
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- In this present study, we found that glucose toxicity occurs severely in ovary and this injury is originated by NF-kappaB way in DM

- Ovarian injury, fibrosis and NF-kappaB immunoexpression are significantly reduced by sunitinib treatment in diabetic rats

- Effects of sunitinib in rat models gives hope to improved treatment of human DM to prevent from premature ovarian failure

- It is highly warranted to continue clinical investigations aiming the discovery of novel targets and mechanisms of sunitinib effect in DM related premature ovarian failure