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Project's Title	In vivo measurement of oxidative stress in diabetic rat retina
Brief Introduction	Diabetic retinopathy is a severe complication of diabetes that develops slowly and is common in patient with diabetes. Diabetic induced oxidative stress is widely considered as the major cause of oxidative damage in diabetic retina. There is no direct method available to measure oxidative stress under in vivo conditions in the retina. We plan to develop a novel method to study oxidative stress under in vivo conditions.
Methodology	Research methodologies: We will use a derivative of reduced fluorescein (from Molecular Probes) as a cell permeant indicator for reactive oxygen species. The dye is carboxy-H2DCFDA a chemically reduced acetylated form of fluorescein. We will inject a small volume of dye into the eye cavity of anesthetized rats. The dye is taken up by cells of the retina. The acetate groups that make the dye cell permeable are removed by intracellular esterases and oxidation of some dye molecules by ROS takes place. Once oxidized the fluorescein derivative becomes fluorescent. The derivative, carboxy-H2DCFDA, carries a negative charge which improves retention of dye within cells. After 10 hours we sacrifice the rats, remove and rinse the retina, and extract the fluorescent dye. We will use known agents which cause oxidative stress in the retina such as lipopolysacharide. We will inject this toxic compound in the vitreous and then measure the production of ROS which will serve as positive control. Expected results: Measurements of ROS will be carried out as described above using 2 and 4 weeks diabetic and their age-matched control rats. We expect that under diabetic conditions oxidative ROS level would be higher which will indicate, in vivo level of ROS in the retina of diabetic rats. The development of this method will give an opportunity to analyze the effect of different drugs in ameliorating oxidative stress under in vivo conditions.
The project is applicable within (months)	8
References	Jakubowski, W., and Bartosz, G. 2,7-dichlorofluorescin oxidation and reactive oxygen species: what does it measure? Cell Biol Int 24:757-760, 2000. Ola MS, Mohd Imtiaz Nawaz, Haseeb A. Khan, Abdullah S. Alhomida

	Neurodegeneration and neuroprotection in diabetic retinopathy Int. J. Mol. Sci. 2012, 13 in press Ola MS, Mohd Imtiaz Nawaz, Marwan Abdulrahman Abouammoh, Abdullah S. Alhomida , Haseeb A. Khan Novel drugs and their targets in the potential treatment of diabetic retinopathy. Medical Science Monitor, submitted Ola MS, Nawaz MI, Siddiquei MM, Al-Amro S, Abu El-Asrar AM. J Diabetes Complications. 2012 Jan-Feb;26(1):56-64. doi: 10.1016/j.jdiacomp.2011.11.004. Epub 2012 Jan 5 .Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. Ola MS, Hosoya K, LaNoue KF.Regulation of glutamate metabolism by hydrocortisone and branched chain keto acids in cultured rat retinal Müller cells (TR-MUL). Neurochem Int. 2011 Oct;59(5):656-63. Epub 2011 Jul 3.