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Dear Editor and Reviewers. We have made the revision of the manuscript. Thank you for the suggestions.

Response to Reviewer 1 Comments

This new manuscript has been carefully examined. The major questions are summarized below: 1) Alloxan is a well-known oxidant (studies began 70 years ago). Oxidative stress is a major potential origin for complications of diabetes mellitus (see for example: Maritim AC et al. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003; 17: 24-38). There is no control group to examine the potential role of the non-diabetic effect of Alloxan on the retina. Are the authors' results a reflection of the toxic effect of Alloxan on the retina of this rat model? 2) There is information about the effects of Alloxan on the retina of rats going well back into the 1960s (i.e. Musacchio ITL, et al. Microaneurysms in the retina of diabetic rats. Lancet 1964; 283(7325): 146).
The authors need to well summarize the limits of the present knowledge of this animal model. It is presently not clear how the authors' experiments provide information to better understand this field.

Response 1: Thank you for the important suggestions .

1) Alloxan is a well-known oxidant (studies began 70 years ago). Oxidative stress is a major potential origin for complications of diabetes mellitus (see for example: Maritim AC et al. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003; 17: 24-38). There is no control group to examine the potential role of the non-diabetic effect of Alloxan on the retina. Are the authors' results a reflection of the toxic effect of Alloxan on the retina of this rat model?

1) Alloxan has a lower direct cytotoxic effect compared with STZ and alloxan models of diabetes enable more accurate timing of selected metabolic events and their pathophysiologic consequences (Like and Rossini, 1976). Moreover, you are fully right, that alloxan produces reactive oxygen species (ROS) with the DNA of the pancreatic islets being one of the targets of ROS (Loreto and Elina, 2009; Malaisse, 1982). However, the addition action of alloxan includes the inhibition of glucokinase, a SH-containing protein essential for insulin secretion induced by glucose (Szkudelski, 2001) and the reducing of glutathione content, which might contribute to its pro-diabetic action. That is why its action could not be limited only by the oxidative action of the compound. According to the literature, Manuelli (1964) has claimed the direct toxic effects of alloxan on the retina, rather than secondary changes from diabetes by

the electron microscopy after administering alloxan intraperitoneally. However, according to our results, the injection of alloxan in the total dose of 30 mg/100 g did not cause any disturbances which could be observed via optical microscopy, that is why we did not provide the information about the control group (there were no changes).

2) There is information about the effects of Alloxan on the retina of rats going well back into the 1960s (i.e. Musacchio ITL, et al. Microaneurysms in the retina of diabetic rats. Lancet 1964; 283(7325): 146).

   Without any doubt, we very much respect the role of Musacchio ITL, et al (1964) among the pioneers in the diabetic retinopathy studies. However, we would like to note, that prof. Musacchio in his works (Musacchio ITL, et al. Microaneurysms in the retina of diabetic rats. Lancet 1964; 283(7325): 146 and Levene, R., Lazzarini-Robertson, A., Foglia, V. G., & Singer, J. (1963). The retina in experimental diabetic rats. Archives of Ophthalmology, 70(2), 253-255.) except the one (Vascular Changes in the Retina of Diabetic Rats , Acta Physiol Lat Amer 11:79, 1961. 9.) mostly used the partial pancreatectomy for inducing the diabetes. Moreover, the experiments lasted up to 27 months and that's why the changes observed could correspond to the later stages of diabetes in humans, while the goal of our experiments was to investigate the pathological changes in the early stages of diabetes.

3) The authors need to well summarize the limits of the present knowledge of this animal model.

   We have added the additional figure and written the Limitation of the Study part.

4) It is presently not clear how the authors' experiments provide information to better understand this field.

   Dear reviewer, the present study was aimed to investigate the pathological changes in the cellular structures of retina and choroidea in the early stages of diabetes. The results of the study, after their translation to the clinic, might improve the current methods diagnosis and treatment, focusing the attention of specialists on the early stages of diabetic complications.

Response to Reviewer 2 Comments

Point 1: Good study. It would have been interesting had the authors continued the study till the stage of diabetic retinopathy and chronicled the changes in the retina and other surrounding structures as they did in the present study. It would also would have been interesting had the authors estimated VEGF, PDEF, cytokines, NO and antioxidants and correlated them with blood glucose levels and changes in the retina and other structures to know which factor(s) are at the root cause of retinopathy. Is the simple increase in plasma glucose is sufficient or glucose-triggered change sin cytokines, VEGF, PDEF, NO, etc., are needed for the development of retinopathy. Such a study would have been clues as to what remedial measures are needed to prevent or manage retinopathy.

Response 1: Thank you for the valuable suggestions. Now we are planning the future studies, and, thanks to you, we shall made the longitudinal research, probably, with the time-points at 6, 12 and 24 months, which should correspond to the later stages of diabetes in humans (or probably, we shall try to establish the high-fat diet/alloxan treated (HFD/STZ) rat model with the observation period of 6-10 weeks). Moreover, we should try to estimate the VEGF, PDEF and the levels of at least 3 (Il-1, TNF and Il-10) cytokines for the better picture. Thank you once again!

Sincerely yours, Alexey P Sarapultsev

Alexey Sarapultsev