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Commentary

Comments on "The improvement Effects of *Gordonia bronchialis* on Male Fertility of Rats with Diabetes Mellitus Induced by Streptozotocin"

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We read with interest a recently published article in *Pharmaceutical Sciences* by Khordadmehr *et al.*, titled "The improvement effects of *Gordonia bronchialis* on male fertility of rats with diabetes mellitus induced by Streprozotocin".[1] They found that 14 days administration of *Gordonia bronchialis* could effectively alleviate blood glucose, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), malondialdehyde (MDA) and improve insulin, superoxide dismutase (SOD) and catalase (CAT) activities in diabetic rats. They also reported that testicular morphology of diabetic rats significantly was improved on days 14 and 21 after the intervention.[1]

It should be noted that these findings might be affected by the quality and reproducibility of experimental design mainly the time of study and sample size used in each of the studied time points.

Diabetes is now one of the most challenging public health issues of our time.[2]A bulk of evidence shows that men with diabetes are at increased risk of low testosterone level, low sexual desire, erectile dysfunction and infertility.[3-6] Therefore, a new research topic has emerged that up to the present time, it has generated a lot of interest among researchers around the world. Performing pre-clinical studies in animal models of diabetes have been widely used to study different aspects of this disease and to pave the way for externalization of particular agents or therapeutic strategies to clinic.[7-10]

Several experimental studies demonstrated that Streptozotocin (STZ) induced diabetes in rodents -particularly in rats- is a suitable model for studying this disease. However, there is evidence showing abnormalities of germ cells in the STZ-induced diabetic rats do not directly correlate with blood glucose levels.[11] Further studies have shown that STZ-induced diabetic model, in the early stage (below 4-week), is not acceptable to study the diabetes-associated spermatogenic dysfunction. It is reasonably demonstrated that spermatogenic dysfunctions like testicular lipid-peroxidation, declined germinal layer of seminiferous tubules and Johnsen's score decrease are attributed to STZ toxicity per se.[12] In the early stage of diabetes induced by STZ, Sertoli cells are affected by STZ (not by hyperglycemia) through over expression of NF-κB (nuclear factor kappa light chain enhancer of activated B cells) and Wnt4 proteins in the testicular tissue.[12] For this reason, most investigations using STZ-diabetic rats have followed for 4-6 weeks,[5] sometimes 8 weeks[13] or diabetic animals have been left untreated for 3-4 weeks before starting the experiment to develop chronic complications of diabetes like testicular impairment.[10,14-16]

The second important point which worth to be considered in the studies using animal models of diabetes is the number of diabetic animals. It is well documented that acute mortality rate within a week after STZ-injection was inversely correlated to animal age as well as weight. Accordingly, the rate of acute mortality in the young animals (6-11 weeks), probably similar to animals used in Khordadmehr *et al.* study, is around 3% in the first week,[17] and according to our previous studies approximately 18% of rats die in the first 4-week following STZ injection.[14] Thus, it is small number (n=5 for each studied point) to ascertain, documented the results and statistical analysis.

Knowledge of the anatomy, physiology, and regulation of the hypothalamic-pituitary-testicular axis is essential for understanding the clinical manifestations, diagnosis, and treatment of the testicular impairments. In adults, the main functions of testes are the production of testosterone and sperm requiring for the maintenance of male characteristics, sexual function, spermatogenesis and fertility.[18] However, in the Khordadmehr *et al.* study none of the parameters related to testicular function such as sperm count and testosterone concentration have been measured.[1]

In the histopathological examination, Johnsen's scoring system was used to evaluate the overall morphological appearance of seminiferous tubules. In the Khordadmehr *et al.* study, the Johnsen's score for diabetic group was reported around 3.5 and 4.5 on the days 14 and 21, respectively. According to the scoring system, scores below than 5, represent seminiferous tubules without spermatozoa.[19] Nevertheless, in the mentioned study, histological micrographs of diabetic groups (Figure5; b-d of Khordadmehr *et al.* study) clearly show the presence of spermatozoa!

Last but not least, another important point in testis histology

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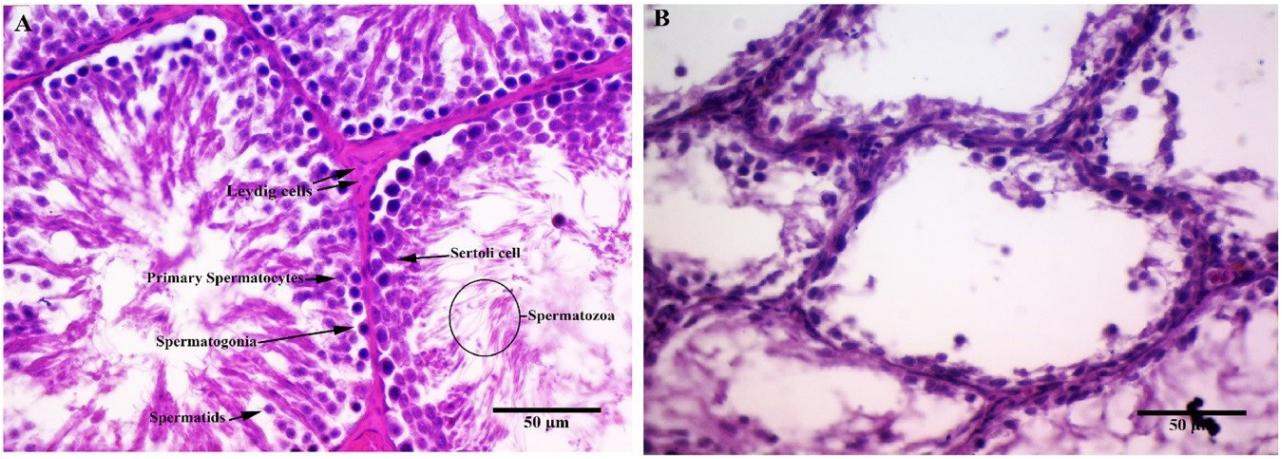

Figure 1. Micrographs show normal (A) and diabetic (B) rat's testes. Hematoxylin and eosin stained (Scale bar: 50 µm).

is seminiferous diameter which is used as an indicator for testicular damage. Surprisingly, in the mentioned study the mean of seminiferous diameter in all groups is reported around 2.87-3.87µm! According to these strange findings, it seems necessary to describe the anatomy of the testis. Briefly, in microscopic view, the testis is a capsulated organ that blood vessels, nerve fibers, lymphatic and genital ducts all enter and leave it. Several septa partition the testis into approximately 250 lobules with different sizes. Each lobule contains 1-4 convoluted seminiferous tubules accounts for 80-90% of the volume of testis and interstitial tissue composed of Leydig cells, mast cells, macrophages, nerves and blood vessels.[20] According to basic histology, a human red blood cell has a diameter approximately 7.5-8.5 µm and in rat, this is around 6.5-7µm.[21] Therefore, blood vessels located between seminiferous tubules logically must have diameters larger than a red blood cell and subsequently seminiferous tubules obviously are greater than interstitial blood vessels. Hence, seminiferous diameters (2.87-3.87 µm) which were reported in Khordadmehr *et al.* study was obviously wrong. Moreover, according to the scale bars of micrographs,[1] it can be easily understood that the diameter of the seminiferous tubules was at least 200 micrometers! Furthermore, the quality of the images was so low that it is impossible to distinguish spermatogonia with spermatocyte, let alone other cells like Sertoli cells. Thus, we provide micrographs of testes from normal and 8-week untreated diabetic rats in which different spermatogenic cells are labeled (Figure 1).

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Conflict of interest

There are neither ethical nor financial conflicts of interest involved in the manuscript.

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