

The Effect of Metformin upon Spermatogenesis in Mice

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ABSTRACT

The effect of the drug Metformin has been studied, employing Mice genome (*mus musculus 2n=40*). Three doses 250, 500 and 1000 mg/ml in addition to the negative control were tested. The results obtained has now been reported for the first time; since a significant increases in sperms 10 fold-increases were obtained. It seems probable that such effect might be caused by enhancement of cell division.

Key words: Metformin, sperm, mice genome.

INTRODUCTION

Spermatogenesis is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testis. This process starts with the mitotic division of the stem cells located close to the basement membrane of the tubules. These cells are called spermatogonial stem cells. The mitotic division of these produces two types of cells. Type A cells replenish the stem cells, and type B cells differentiate into spermatocytes. The primary spermatocyte divides mitotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two equal haploid spermatids by Meiosis II. The spermatids are transformed into spermatozoa (sperm) by the process called Spermatogenesis. These develop into mature spermatozoa, also known as sperm cells (Sharma *et al.*, 2018). Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa.

Spermatozoa are the mature male gametes in many sexually reproducing organisms. Thus, spermatogenesis is the male version of gametogenesis, of which the female equivalent is oogenesis. In mammals it occurs in the seminiferous tubules of the male testes in a stepwise fashion. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. DNA methylation and histone modification have been implicated in the regulation of this process (Song *et al.*, 2011) It starts at puberty and usually continues uninterrupted until death, although a slight decrease can be discerned in the quantity of produced sperm with increase in age.

Metformin is primarily used for the treatment of type 2 diabetes mellitus, particularly in obese patients.

Metformin has been shown to reduce diabetes mortality and complications by thirty percent compared to insulin, glibenclamide and chlorpropamide. (Rafieian-Kopaei and Baradaran 2013).

MATERIALS AND METHODS

The experimental work of this study was carried out at Laboratory of Genetic Toxicology, Faculty of Agriculture, Alexandria University, Department of Genetics, The present investigation was planned to: Investigate the effect of metformin upon germinal line in mice. The drug was locally purchased and used in three doses 250,500 and 1000 mg per kg body weight.

Mice (*Mus musculus 2n=40*) were employed and each mouse had taken the proper dose once a day for a 30 days. Cell collection was carried out according to (Pary and Pary 1984).

Geimsa stain was used and cells were microscopically investigated, counted and photographed where needed.

Drug used:

Metformin (dimethylbiguanide) features as a current first-line pharmacological treatment for type 2 diabetes (T2D) in almost all guidelines and recommendations worldwide. It has been known that the antihyperglycemic effect of metformin is mainly due to the inhibition of hepatic glucose output, and therefore, the liver is presumably the primary site of metformin function.

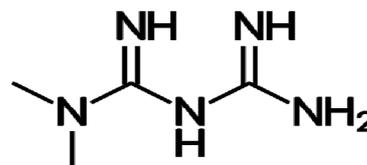


Figure 1.The chemical component of metformin

Formula of metformin	C ₄ H ₁₁ N ₅
Molar mass	129.16364 g/mol

However, in this issue of Diabetes Care, Fineman and colleagues (Buse *et al.*, 2016) demonstrated surprising results from their clinical trials that suggest the primary effect of metformin resides in the human gut.

Metformin, marketed under the trade name **Glucophage** among others, is the first-line medication

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for the treatment of type 2 diabetes (Maruthur et al., 2016) particularly in people who are overweight.

Experimental animal model:

A total of 60 male albino mice, *Mus musculus*, (25-30g) were obtained from the Medical Research Institute Alexandria University. Animals were kept in plastic cages (ten animals per cage) covered with metallic grids in a room maintained at proper environmental conditions of temperature 25°C and humidity 50% with a 12-hours light-dark cycle. The animals were acclimatized for 4 weeks before the start of the experiment and they were given free access food and water.

Glucophage experiment design:

Sixty male albino mice were divided randomly into four groups. The duration for all experimental groups was 30 days (Xie et al., 2000).

- Group 1:** normal control group of fifteen mice. These mice did not receive any treatment during the experiment stages.
- Group 2:** Glucophage commercial formula for Metformin, was treated for this group and fifteen mice had received one tablet of Glucophage dissolved in 100 ml, double distilled water, respectively. They had received 250 µl daily in oral dose.
- Group 3:** Glucophage commercial formula for Metformin, was treated for this group and fifteen mice had received one tablet of Glucophage dissolved in 100 ml, double distilled water, respectively. They had received 500 µl daily in oral dose.
- Group 4:** Glucophage commercial formula for Metformin, was treated for this group and fifteen mice had received one tablet of Glucophage dissolved in 100 ml, double distilled water, respectively. They had received 1000 µl daily in oral dose.

Analysis of mice primary spermatocytes

Fifteen male mice were used for each dose. Doses had orally given one time daily for a 30 days. Ten days after the last dose the animals were killed by cervical dislocation. The used procedure follows basically the description given by Oud *et al.*, (1979); Adler (1984); Seehy and Osman (1989) and Seehy M. (2007).

Tissue sampling:

The testes were removed by making an incision into the scrotum and fat tissue was cleaned. The tunica were removed, transferred the tubules to a small Petri dish containing a piece of fly mesh and 3-4 ml of 2.2 % trisodium citrate. The tubules were cut up with forceps

several times, and then they were mashed on the fly mesh with flat-top forceps. The fluid containing the cells was transferred to 12 X 100 mm round-bottom centrifuge tubes, centrifuged at 1000 rpm for 5 min. supernatant was completely discarded. The hypotonic solution (1 % trisodium citrate) was slowly added and centrifuged, after 15-20 min, and then the cells were fixed in (methanol: glacial acetic acid, 3:1). The fixative was changed twice after 10 min for each by centrifugation between changes.

Staining

The slides were stained for at least 10 min using 10 % Giemsa (PH6.8), washed air-dried and examined microscopically.

RERSULTS AND DISCUSSION

As shown table 1 it was found that the total number of cells was found to be 2500, 5000, 12000 and 1000 after treatment with 250, 500 and 1000 doses and control, respectively.

The relative percent of cells to the control group % at the level of this study was 2.5%, 5% and 12%.

It is clear that the drug metformin was proven to be capable in inducing significant increases in animal sperms, and accordingly in animal fertility.

Attia et al, (2009) reported metformin was neither genotoxic nor cytotoxic for the rats in all groups at all tested doses. Moreover, metformin significantly reduced the diabetes-induced genomic instability and cell proliferation changes in somatic and germinal cells in a dose-dependent manner (2500, 500, >100mg/kg). In addition, diabetes induced marked biochemical alterations characteristic of oxidative stress including, enhanced lipid peroxidation and reduction in the reduced glutathione level. Treatment with metformin ameliorated these biochemical markers. In conclusion, metformin is a non-genotoxic or cytotoxic compound and may protect from genomic instability induced by hyperglycemia. Apart from its well-known anti-diabetic effect, the antigenotoxic effect of metformin could be possibly ascribed to its radical scavenger effect that modulated the genomic instability responses and cell proliferation changes induced by hyperglycemia.

Metformin, an old antidiabetes drug, may inhibit prostate intraepithelial neoplasia transforming to cancer lesion via reducing c-MYC, an 'old' overexpressed oncogene. This study explores chemopreventive efficacy of metformin in prostate cancer and its link to cMYC *in vitro* and *in vivo*. Akinyeke et al., (2013).

Table 1. shows the effect of different doses of metformin upon number of sperms after treatment for 30 days

Dose mg/ml	Number of cells	Percent; relative to negative control %
250	2500	2.5
500	5000	5
1000	12000	12
NC	1000	1

*NC : Negative Control.

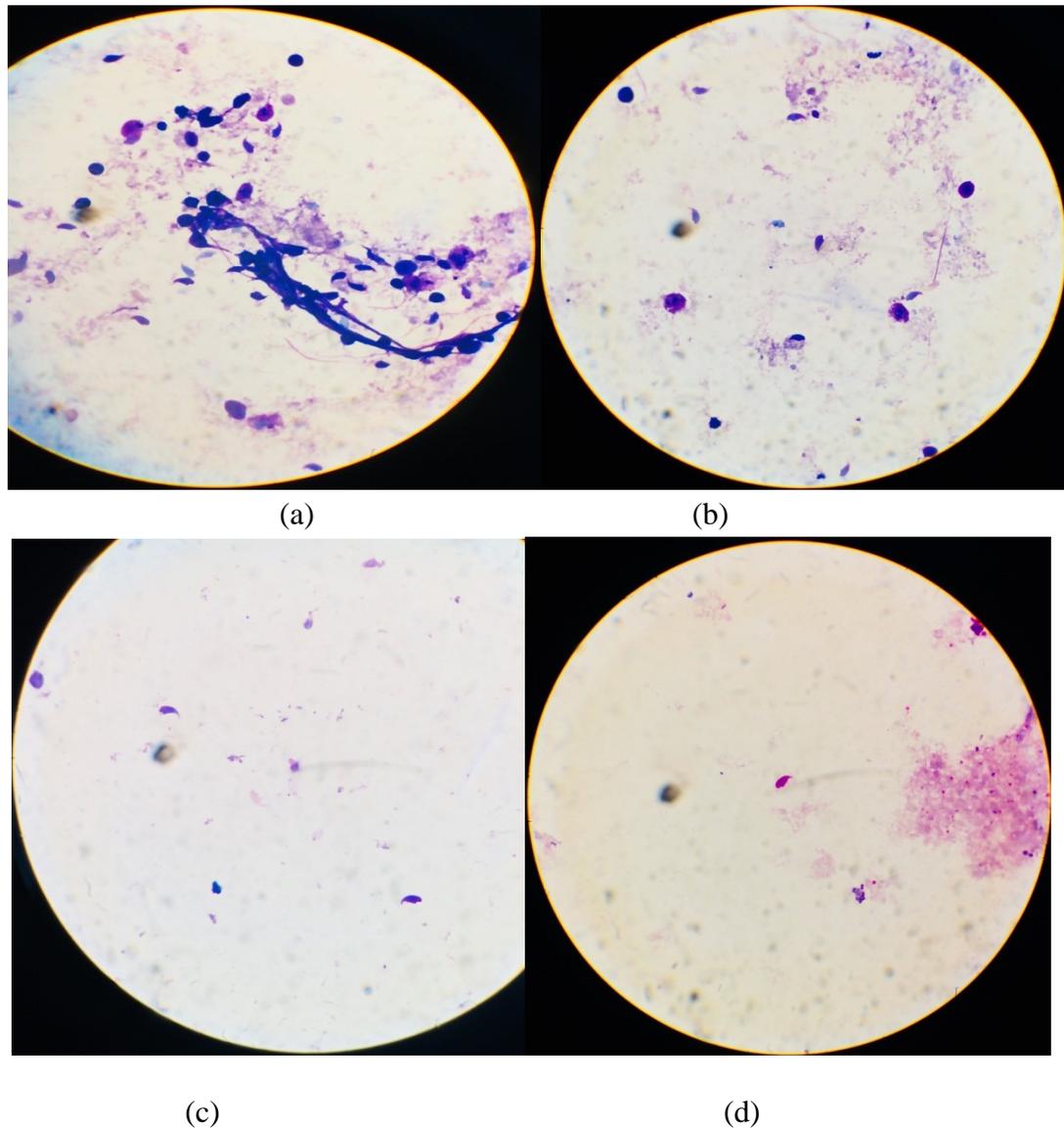


Fig.2. Photomicrograph showing. (a) High increases in number of sperm due to the 1000mg/ml dose. (b) The effect of dose 500mg/ml. (c) The effect of 250mg/ml dose. (d) The negative control

Therefore, metformin may be a potent candidate for chemoprevention of liver tumorigenesis in patients with obesity or diabetes.(Ohno et al.,2015)

The conclusion came from the present investigation is the following:

The effect of metformin has now been observed for the first time by Aalaa Ayyad and Mohamed El-Seehy

that the drug metformin is capable to increase mammalian fertility One can conclude that this effect is caused by its capability to enhance cellular division.

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المخلص العربي

تأثير عقار المتفورمين علي تكوين الحيوانات المنوية في الفئران المعملية

الاء عياد و محمد الصيحي

وقد جاءت نتائج البحث حيث تم ملاحظة زيادة عدد الحيوانات المنوية بحيث كانت ٢,٥ ضعف المجموعة الضابطة و٥ أمثال المجموعة الضابطة ثم ١٢ ضعف بعد المعاملة بالجرعة الأعلى.

هدف البحث الحالي لدراسة أثر عقار المتفورمين الذي يستخدم في علاج مرض السكر من النوع الثاني وأثره على الخصوبة وتم توظيف الفئران المعملية البيضاء باستخدام ٦٠ فأر وقد قسمت الفئران لاربعة مجموعات المجموعة الاولى تركيز الجرعة ١٠٠٠ ملجم/ ٦٠ كجم و ٥٠٠ و ٢٥٠ والمجموعة الرابعة ككنترول ولم تاخذ اي جرعات.