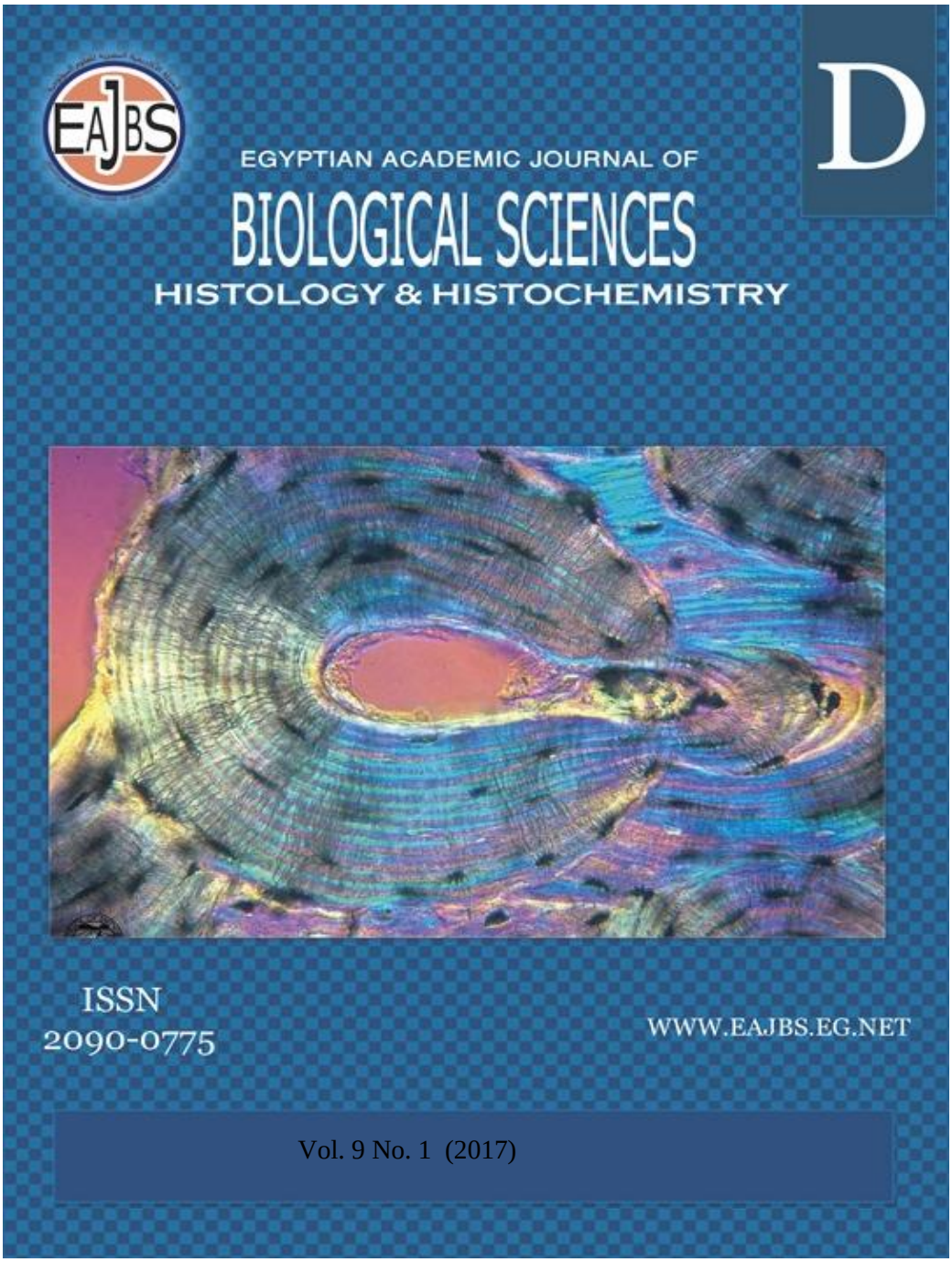


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# Effects of 5-Fluorouracil on Testes Histology and Sperm Morphology Assay in Mice

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**ABSTRACT**

Pathological effects of the chemotherapeutic agent 5-FU on mice testes and their sperm morphology were studied. Animal were injected intraperitoneal with small dose of 5-FU (5, 10 and 15 mg/kg) given for five consecutive days. Treated mice were left for 35 days before taking the histological and sperm samples. Immature germ cells sloughing, spermatid haloapperance and multinucleated giant cells were detected in the testes of the treated mice. All used 5-FU doses (5, 10 and 15 mg/kg b.wt) significantly ($p<0.05$) increased the numbers of morphologically abnormal sperms compared with the control. However, there were no significant statistical differences between the three doses of 5-FU. Also, it was found that the mean values of the morphologically abnormal sperms increased as the drug doses increased.

## INTRODUCTION

5-FU is used to treat colorectal cancer as well as other kinds of cancer (Longley *et al*, 2003). Its cytotoxic effects on cancer cells exerts through inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA (Longley *et al*, 2003).

Male gonadotoxicity of 5-FU were manifested as sloughing of the seminiferous epithelium and formation of giant cells. Narayana *et al*. (2000) using single dose of 5-FU (10, 50 and 100 mg/kg, i.p.) reported sloughing of seminiferous epithelium at all doses. Only the highest dose (100 mg/kg) resulted in the formation of giant cells.

El-Sayyad *et al*. (2013) treated male albino rats by 5-fluorouracil (i.p. 20 mg/kg BW, every other day for 30 days) observed sclerosis of seminiferous tubules and formation of multinucleated giant cells.

Due to the constant production and development of sperm they are a main target for chemotherapy. It is generally accepted that sperm morphology evaluation is a good method to estimate sperm-fertilising ability (Liu and Baker, 1990, 1992; Esterhuizen *et al*., 2001 and Abu Hassan Abu *et al*., 2012). Misshapen mouse spermatozoa are seldom fertilizing oocytes either *in vivo* or *in vitro* (Krzanowska and Lorenc, 1983).

In turn, present study aimed to investigate the effects of 5-FU low doses (i.p. 5, 10 and 15 mg/kg B.W.) on male mice testes histology and sperm morphology.

## MATERIALS AND METHODS

**Drug:**

Neoflur (5-FU) is supplied as a 250 mg dissolved in 5 ml water; each 1 ml contains 50 mg 5-FU. 5-FU was diluted in injectable water for all doses and used immediately after dilution.

**Experimental animals:**

Male adult albino mice (*Mus musculus*) obtained from National Research Centre, Cairo, was used in all experiments conducted in this study. At the beginning of each experiment the mice age between (7 to 10 weeks) and weighing between (20-30 gm) were kept in cages under standard conditions, i.e. a well ventilated room and a controlled regimen of fluorescent light (light for 12 hours and dark for 12 hours) at the Animal House of Zoology department, Faculty of Science, Suez Canal University. Mice were housed in plastic cages, wire topped with sawdust bedding. The sawdust bedding of the mouse boxes was changed weekly and the cages were cleaned and sterilized. Mice were fed on standard diet and tap water was given ad libitum. They were acclimatized to their place for one week before the experiment. Animals were randomly allocated into four separate experiments. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources (Suez Canal University, Egypt) and all efforts were made to reduce the number of animals used and their suffering.

**Experimental design:**

Animals were divided into 4 groups (a control group and three treated ones) of 5 male mice each. Three groups were injected intraperitoneally with 5-FU (5, 10 and 15 mg/kg) for five consecutive days at intervals of 24 hr. Mice of the control group were injected with injectable water. All animals were sacrificed on day 35 following the last injection. Post treatment sampling at 35 days was chosen for the study to allow the germ cells, which were exposed at late spermatogonial stage to the drug (5-FU) to reach the caudal epididymis.

**Histological preparation:**

Animals were anesthetized using chloroform, dissected and tissues samples of testes were taken out. Tissue samples were fixed in Bouins fluid for 48 hr. The fixative was changed twice during 48 hr. The samples were then washed several times in 70% ethyl alcohol to remove the excess fixative, dehydrated using ascending series of alcohols (80%, 90%, 100% and 100%), cleared in Terpineol and embedded in paraffin wax. Tissue blocks were sectioned at 5 µ thickness and stained with Harris Haematoxylin and Eosin (Mallory, 1944).

**Sperm sampling and staining:**

The epididymides were excised and minced in 1 ml of 0.9% physiological saline. The contents were gently pipetted or squeezed five to six times up and down in a 5 ml pipette. The sperm solution was filtered through a nylon cloth to remove tissue fragments. A small drop of the cell suspension was put on the end of a clean slide and spread by pulling the material behind a clean glass cover held at an angle of 45 degrees. The slides were air dried without fixation for about 24 hr. Slides were stained with 1% Eosin-Y (aqueous) for 30 minutes followed by two rinses in distilled water. Slides were then left to air dry and cleared in two changes of Xylene, 5 minutes each. Five slides were prepared for each mouse. Sperm smears were examined by light microscopy. For each mouse, 800 sperms were examined and morphological abnormalities of sperm head and tail were recorded according to the criteria of EL-Nahas *et al*. (1989). Abnormal sperm morphology is classified as defects in the head, midpieces and tail (Burruel *et al*., 1996).

**Statistical analysis:**

The statistical analysis was carried out using SPSS Statistical Package, version 13 for Windows (SPSS Inc., Chicago, IL, USA). The results were analyzed by performing ANOVA and Tukey's multiple comparison tests with significance level was set at $p < 0.05$.

## RESULT

Testes of control mice were histological normal (Fig. 1). Testes of treated mice showed histological altered seminiferous tubules, at all doses. This alteration was in form of sloughing (loss) of immature germ cells into the tubular lumen. Some of the tubules showed haloapperance in the round spermatids. Spermatids of some tubules were observed forming round multinucleated giant cells (Fig. 2).

5-FU treatment affected the percentages of morphologically abnormal sperms at all of the three tested doses. Various morphological sperm abnormalities were observed in control and treated animals. Table (1) showed the percentages of normal and abnormal sperms in the control and treated groups. Percentages of morphologically abnormal sperms in the control and treated groups were 15.1%, 39.4%, 41% and 47.2%, respectively.

Table 1: The means and the percentages of abnormal sperms count.

| | **Dose mg/kg** | **Mice number** | **Number of dead mice** | **Mean of abnormal sperms (out of 800 sperm per mouse) ± S.E.** | **Percentage of the abnormal sperms** |
|---|---|---|---|---|---|
| **Control** | 0 | 10 | 0 | 121±21 | **15.1%** |
| **5-FU treated groups** | 5 | 9 | 1 | 315±44 | **39.4%** |
| | 10 | 8 | 2 | 326±50 | **41%** |
| | 15 | 6 | 4 | 378±40 | **47.2%** |

Morphological abnormalities of mice sperms induced by 5-FU treatment, in the present study, were grossly headed sperms, quasi-normal headed sperms, angular midpiece sperms and bended tailed sperms (Fig. 3A-C).

Figure (4) showed graphically the mean values of morphologically abnormal sperms for the four studied groups while Figure (5) showed the linear trendline for the four studied groups which revealed that the mean values of the morphologically abnormal sperms increased as the drug doses increased.

The ANOVA test showed that the statistical differences between the control group and the 5-FU treated groups (5, 10 and 15 mg/kg) were statistically highly significant ($p= 0.000267$ at the level of significance of 0.05). The multiple comparisons between the four studied groups using Post Hoc Tukey test revealed that the statistical differences between the control and each of the studied drug doses (5, 10 and 15 mg/kg) were highly significant ($p = 0.004$, 0.003 and 0.001 respectively at level of significance of 0.05). However, the statistical differences among the drug doses 5, 10 and 15 mg/kg were insignificant.

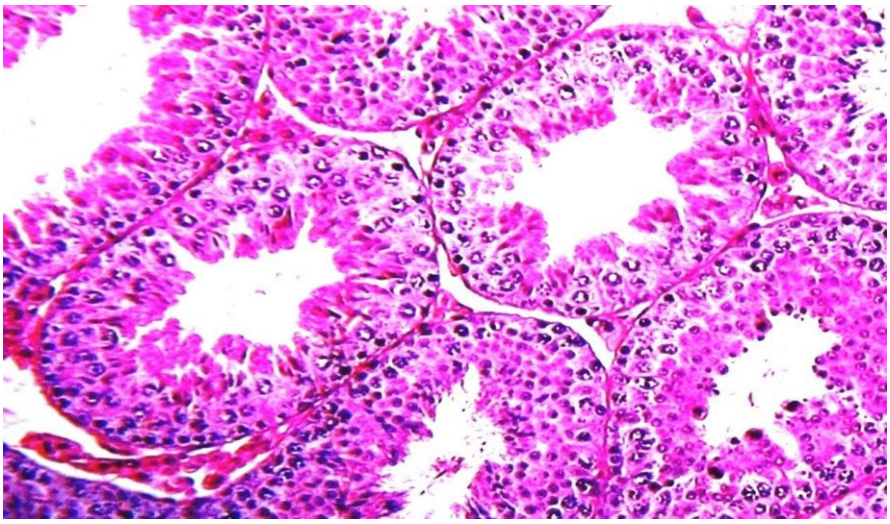

Fig.1: Testes section of control mice receiving injectable water and left for 35 days showing normal seminiferous tubules (HE, X 200).

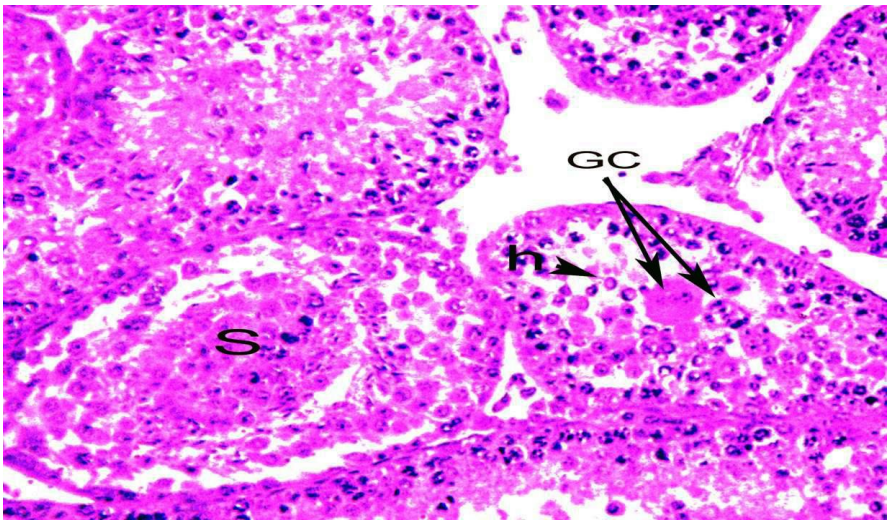


Fig. 2: Testes section of mice injected intrapertoneally with 15 mg/kg 5-FU for 5 consecutive days and left for 35 days showing sloughing (S) of immature germ cells into the tubular lumen. Some of the tubules showed haloapperance in the round spermatids (h). Spermatids of some tubules were observed forming round multinucleated giant cells (GC) (HE, X 200).

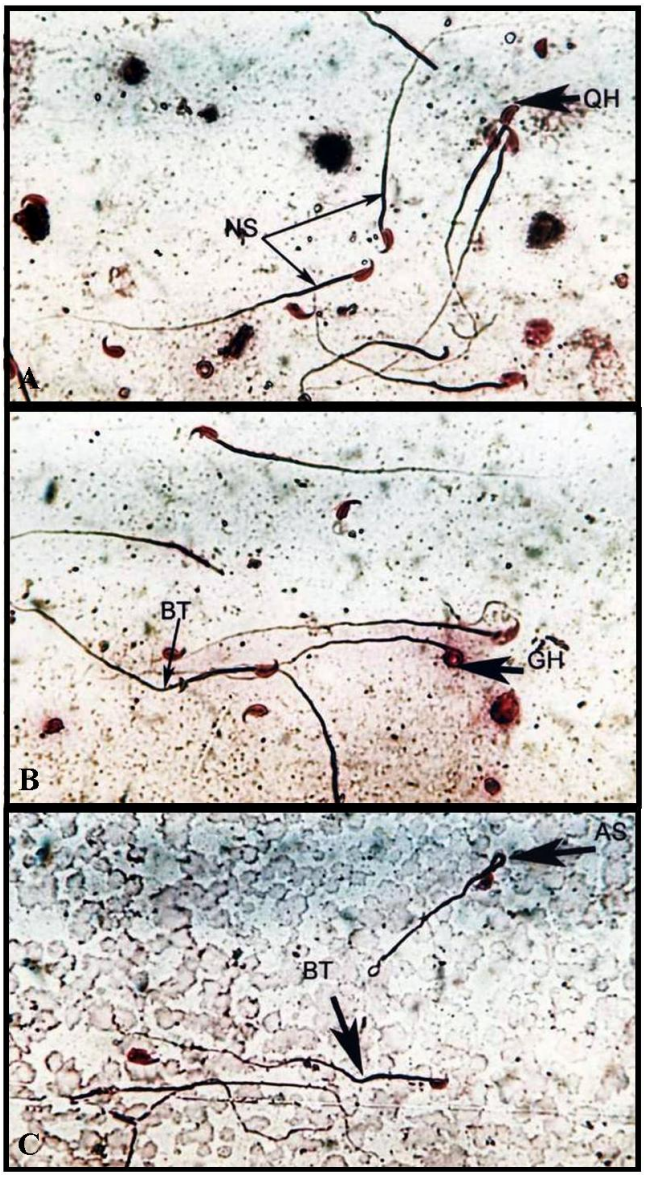


Fig. (3A-C): sperm smears of treated mice. A) Sperm smear showing quasi-normal head (QH) and normal sperm (NS); B) Sperm smear viewing grossly abnormal head (GH) and bended tail (BT) and C) Sperm smear revealing angular midpiece sperm (AS) and bended tail sperm (BT). Eosin stain, 400X.

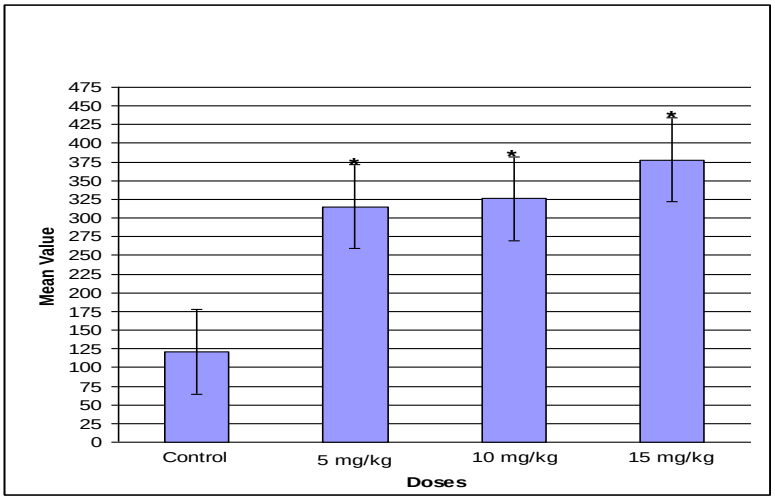


Fig. 4: Mean values of morphologically abnormal sperms out of 800 sperms per mouse in control group as well as treated groups.
(*) represented statistically significant differences between the control group and the 5-FU treated groups (5, 10 and 15 mg/kg).

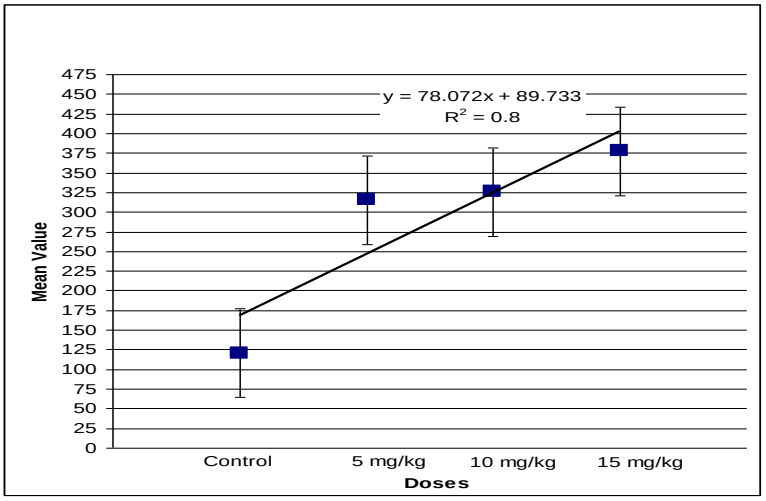


Fig. 5: The linear trendline of the abnormal sperms for the four studied groups.

## DISCUSSION

At all used doses, the testes of the treated mice showed some histologically altered seminiferous tubules, whereas other seminiferous tubules were not affected. The fact that some of the seminiferous tubules were altered and other tubules were not, had been supported by Meistrich *et al*. (1982) who mentioned that the intracellular half-life of the drug is 7 to 9 days so that the stem cells may be triggered into cycle by administration of 5-FU while the drug is still active. Histologically altered seminiferous tubules showed sloughing of the germinal epithelium. This is similar to studies in rats by D'Souza and Narayana (2001) who found sloughing of the germinal epithelium in the lumen after injecting 10, 50 and 100 mg/kg 5-FU intraperitoneally.

In the present study, multinucleated giant cells and haloapearence spermatids observed in seminiferous tubulus of the treated mice are in agreement with the findings of Narayana *et al*. (2000) who found multinucleated giant cells in the seminiferous tubulus lumen after injecting 100 mg/kg 5-FU intraperitoneally.

Regarding the results of sperm morphology assay, this work revealed a statistically significant increase in the percentage of morphologically abnormal sperms at all used doses. Such percentages were 39.5%, 41% and 47.2% for 5, 10 and 15 mg/kg 5-FU respectively, as compared with 15.1% for the control animals. The differences between the control group and the treated groups were statistically significant ($p<0.05$), but the differences in the abnormal sperm count between the treated groups turned to be statistically insignificant. This is not in agreement with the findings of Choudhury *et al*. (2002) who used a single intraperitoneal injection of 5, 10 and 15 mg/kg 5-FU and carry out sperm morphology assay at week 8 post-treatment. He reported high percentages of abnormal sperm, but were not statistically significant. He attributed this to the gradual decline in the transmission of the induced cytogenetic toxic effects of 5-FU from spermatogonia to sperm, due to gradual elimination of the grossly affected spermatogonial cells during the course of spermatogenesis.

Morphological abnormalities of mice sperms induced by 5-FU treatment, in the present study, were grossly headed sperms, quasi-normal headed sperms, angular midpiece sperms and bended tailed sperms.

According to Wyrobeck (1984) the significant increase in the number of morphologically abnormal sperm has been associated with infertility. In the three used doses (5, 10 and 15 mg/kg) of 5-FU quasi-normal headed sperms were observed in the examined sperm smears. Quasi-normal head defects do not seem to affect the motility of spermatozoa but significantly reduce the *in vitro* and the *in vivo* fertilizing capacity (Jeyendran *et al.*, 1986).

The quasi-normal head may be due to the action of 5-FU on the genes responsible for expression of acrosomes characteristics (Topham, 1980). Sperms with abnormal tail either with coiled tail or bended tail were observed. These abnormalities affect the motility of the sperm. Menkeld *et al*. (1990) related tail coiling to sperm aging. Sperms with bended midpiece were recorded. Menkeld *et al*. (1990) suggested that bended midpiece had grown from wrong centriole. Sperm cell morphology is genetically controlled by numerous autosomal and sex linked genes (Krazanowska, 1976). Hence, formation of abnormal sperm population in the present study is very likely due to the mutagenic effects of 5-FU on the specific gene loci of germ cell chromosomes involved in the maintenance of normal sperm structure. These mutagenic effects of 5-FU are primarilary caused by the direct cytotoxicity of germ cells during spermatogenesis. The cytotoxicity is caused by the antimetabolic activity of 5-FU through the inhibition of thymidylate synthestase and the erroneous incorporation into RNA and DNA (O`Dwyer *et al*., 1987 and Pinedo and Peters, 1988).

We conclude that even small dose of 5-FU affect the patient fertility by disturbing the testes histology and sperm morphology.

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## ARABIC SUMMARY

**تأثيرات ٥- فلوروبوراسيل على التركيب النسيجي للخصيتين والشكل الظاهري للحيوانات المنوية في الفئران**

**هبه ناجح جاد الحق[١] وطارق إبراهيم سعد معوض[١] وجمال عبد العاطي فايز[٢]**
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٢- جامعة قناة السويس – كلية الطب – قسم الباثولوجي – الإسماعيلية – مصر.

تمت دراسة الآثار المرضية للعامل العلاجي الكيميائي 5-FU على الخصيتين في الفئران ومورفولوجية الحيوانات المنوية. تم حقن الحيوانات داخل التجويف البروتوني بجرعة صغيرة من 5-FU (٥ و ١٠ و ١٥ ملج / كلج) لمدة خمسة أيام متتالية. تركت الفئران التي عولجت لمدة ٣٥ يوما قبل أخذ العينات النسيجية والحيوانات المنوية. لوحظ وجود خلايا جرثومية غير ناضجة متساقطة وكذلك haloapperance spermatids والخلايا العملاقة متعددة النوى في خصي الفئران المعالجة. كل جرعات 5-FU المستخدمة (٥ و ١٠ و ١٥ ملج / كلج) قد أدت لزيادة أعداد الحيوانات المنوية ذات الأشكال غير الطبيعية زيادة معنوية ($p<0.05$) مقارنة بالكنترول. ومع ذلك، لم تكن هناك فروق ذات دلالة إحصائية معنوية بين الجرعات الثلاث المستخدمة من 5-FU. كما وجد أن القيم المتوسطة للحيوانات المنوية ذات الأشكال غير الطبيعية زادت مع زيادة جرعات الدواء.