# نتائج موجبة لأختبار الروزبنكال في الخيول

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## ملاحظات قصيرة

لوحظت حالات متفرقة في مجموعة من الخيول شملت ناسور الحارك، التهاب المفاصل وتورم كيس الصفن. سحبت عينات دم من بعض هذه الحالات ومن خيول تبدو سليمة لقياس خضاب الدم وأجراء اختبار الروزبنكال ( Rose Bengal test) . كذلك تم سحب نموذج من السائل في كيس الصفن. أظهرت الفحوصات أن مستوى خضاب الدم كان ضمن الحدود الطبيعة (11.6- 16.2)غم/ 100سم$^3$ . وان خمسة خيول من عشرة (50% ) أظهرت نتيجة موجبة لأختبار الروزبنكال وكانت من الجنسين وبأعمار مختلفة ، ولم تقتصر على الخيول التي أظهرت ناسور أو تورم . ولم تعزل جراثيم من السائل المصلي الرائق والحاوي على كريات دم حمراء وخلايا قيحية والمسحوب من كيس الصفن لأحد الخيول .

# HISTOLOGICAL CHANGES IN THE TESTI OF PURE INBRED SWISS MICE INDUCED BY PERMETHRIN INSECTICIDE

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## SUMMARY

Permethrin, is a synthetic insecticide of appreciably low mammalian toxicity, it is especially useful for household purposes. This work aimed to study the gonadotoxic effect of this insecticide in Swiss mice, using two oral doses of 2000 and 3000 mg/kg b. w. for 30 days. The daily dose was 1.66 mg/kg and 2.5 mg/kg of active ingredient of permethrin for group I and group II respectively. The effect was determined by counting the percentage of damaged seminiferous tubules, their average diameter, and the spermatogenesis rate, also the changes in body weight and in testes-to-body weight ratio. Results showed that the insecticide caused significant reduction in body weight, increase in testes weight, as

well as testes-to-body weight ratio, seminiferous tubules in treated animals were found to increase in diameter showing low degrees of damage. Nevertheless, spermatogenic activity was almost normal but in descending order, and Leydig cells remained unaffected. It was concluded that permethrin, has a slight gonadotoxic effect at the doses used which may not really affect the fertility of animals at the doses used.

## INTRODUCTION

Permethrin is a selective, photostable, organic insecticide of the synthetic pyrethroids group, it is composed of 2:3 cis-/trans- isomeric ratio (1). As insecticide it is particularly useful for household purposes, therefore it represent an indoor air pollutant as well as a food contaminant (2). In addition to its use for veterinary purposes (3), agricultural uses are not uncommon, particularly for the protection of the stored grains (4,5). Synthetic pyrethroids in general are neurotoxins (6,7) though this toxicity is relatively low to mammals (8,9) and man (10).

Oral $LD_{50}$ values vary considerably with animal species, vehicle used for administration and cis:trans isomeric ratio affect (11). Aqueous suspensions produces the least toxic effects, showing oral $LD_{50}$ values in rats and mice ranging from 3000 to >4000 mg/Kg b. w. Nevertheless, corn oil is accepted as a standard vehicle for oral administration (12,13).

Toxicity evaluation of permethrin is performed in mice and rats at varying dose levels up to 10000 mg/kg diet for duration ranging from 2 to 26 weeks (13). Histological changes related to permethrin administration were observed in liver, kidney, and lung in mice at a dose of 2500mg/kg diet (14). Scarce published works are available concerning the effects of permethrin on reproductive tissues. Therefore this study was carried out to investigate the possible gonadotoxic effects of this insecticide on the testicular tissues in Swiss mice.

## MATERIALS AND METHODS

Thirty sexually mature laboratory bred, male Swiss mice of an average body weight of 25±3gm were used. They were randomly divided into three groups of ten mice each. Group I &II, received daily doses of 2000 and 3000 mg/kg b. w of permethrin (95%) respectively,

dissolved in corn oil, orally by means of a stomach tube consecutively for 30 days, so that each mouse in group I received daily doses of 1.66 mg/kg of the active ingredient of permethrin and each mouse in group II received 2.5 mg/kg b. w./day of the active ingredient. Group III was considered as control group receiving the same volume of the vehicle (0.1ml corn oil) daily.

All animals were kept under the same conditions. Diet and water were allowed *ad libitum*. Body weight for all animals was recorded at three - days intervals. Animals were sacrificed 24 hr after the last dose. Testis were removed, weighted, fixed in Bouin's fluid and processed in a series of graded ethanol for dehydration. Paraffin sections were cut out at 6 micrometer thickness, stained with Harris hematoxylin and eosin stain, and examined by light microscope.Five consecutive sections from each single testis were examined. The effect of permethrin was determined through the following indices: the percentage of damaged seminiferous tubules in each section, the average diameter of the seminiferous tubules based on 15 regular shaped tubules as well as any deviation from the normal histological picture of testicular tissue. Data were analyzed using F-test.

# RESULTS

Signs of intoxication in the form of tremors, hyperactivity were noticed especially in group II. No mortality was reported throughout the study period, however significant reduction in body weight was noticed in both treated groups (I&II). Loss in body weight of group I was 20.09% of the starting weight, the decrease was more marked in group II reaching 23.56% of the starting weight. The reduction becomes statistically significant at ($P<0.05$), particularly at the second week of dosing, and continued to be more significant ($p<0.01$) in the third week and continued to the end of the study period as compared with control group. While no significant difference in body weight was noticed when comparing both treated groups (group I&II) together. On the other hand, animals of control group showed slight gradual decrease in body weight reaching 3.55% of the original at the end of the experiment (Table 1).

**Table 1** Changes in body weight of all animals of three groups as taken at three-day intervals ± Standard Error, and the percentage of decrease in body weight.

| No. of animal | Control group | | Group I | | Group II | |
|---|---|---|---|---|---|---|
| | Average b.w gm ±S.E. | % of decrease | Average b.w gm±S.E. | % of decrease | Average b.w gm ±S.E. | % of decrease |
| 1 | 26.71±0.316 | 2.7 | 25.83±0.274 | 13.04 | 26.35±0.433 | 19.35 |
| 2 | 27.67±0.315 | 6.25 | 24.85±0.474 | 11.8 | 26.41±0.174 | 27.37 |
| 3 | 26.53±0.343 | 1.88 | 24.26±0.544 | 17.44 | 24.29±0.587 | 15.23 |
| 4 | 26.46±0.285 | 2.98 | 23.60±0.408* | 26 | 24.76±0.696* | 14.65 |
| 5 | 26.60±0.275 | 2.57 | 22.88±0.418** | 21.9 | 23.09±0.706** | 28.71 |
| 6 | 26.34±0.352 | 8.75 | 22.53±0.556** | 22.28 | 23.23±1.001** | 29.73 |
| 7 | 25.90±0.314 | 1.18 | 21.59±0.438** | 17.5 | 22.02±0.689** | 21.22 |
| 8 | 25.59±0.279 | 2.62 | 21.09±0.804** | 19.76 | 22.07±1.315** | 34.36 |
| 9 | 25.70±0.301 | 3.1 | 20.35±0.581** | 27.03 | 21.81±0.859** | 23.42 |
| 10 | 25.75±0.227 | 3.46 | 20.16±0.549** | 24.24 | 20.24±0.647** | 21.57 |
| Mean ±S.E. | 26.33± 0.198 | 3.55 ±0.844 | 22.71 ± 0.610 | 20.09 ± 1.278 | 23.43 ± 0.799 | 23.56 ± 1.566 |

*Significant at P< 0.05; ** Significant at P<0.01.
S.E.= Standard Error

The results were showed an increase in testes weight (in mg), as well as testes-to-body weight ratio (calculated as mg/gm respectively) was remarkable in both treated groups (I&II) (taking in account body weight reduction), the ratio in control group was found 6.82 while that of group I was 8.83 and of group II was 9.01 . The increase was statistically significant as shown in (Table 2).

**Table 2**: The testes weight of the animals of the three groups in (mg), and testes-to- body weight ratio (mg/gm).

| No of animal | Control Group | | Group I | | Group II | |
|---|---|---|---|---|---|---|
| | Testis weight | Ratio | Testis weight | Ratio | Testis weight | Ratio |
| 1 | 175.3 | 7.01 | 179 | 8.14 | 180 | 8.78 |
| 2 | 178.4 | 6.59 | 179 | 7.75 | 181.3 | 9.07 |
| 3 | 177.5 | 6.82 | 179 | 8.4 | 182 | 7.38 |
| 4 | 177 | 6.8 | 178 | 8.81 | 181.7 | 9.66 |
| 5 | 177.3 | 6.69 | 175 | 8.93 | 181 | 9.12 |
| 6 | 174.2 | 6.96 | 174.2 | 10.12 | 181.2 | 8.97 |
| 7 | 174.8 | 6.90 | 175 | 9.72 | 179.4 | 10.55 |
| 8 | 175 | 6.73 | 174.8 | 8.78 | 178.5 | 9.15 |
| 9 | 174 | 6.96 | 178 | 8.76 | 179.6 | 8.45 |
| 10 | 174 | 6.76 | 177 | 8.85 | 179 | 8.95 |
| Mean ±S.E. | 175.75 ±o.519 | 6.82 ±0.043 | 176.8* ±0.787 | 8.83** ±0.218 | 180.27** ±0.386 | 9.01** ±0.255 |

* Significant at P<0.05. ** Significant at P< 0.01. S.E. = Standard Error

Testis in control group did not show any histological changes (Fig .1), while permethrin-related changes were observed in both treated groups (I&II). Damage in the seminiferous tubules was invariably present in all sections examined. Both damaged and intact tubules could be noticed lying adjacent to each other within the same field. However, spermatogenic

activity was almost normal but in descending order, the tubules carried less number of spermatogenic cells in comparison with control (Fig 2&3). The percentages of the damaged tubules are shown in (Table 3). It did not exceed 1.28% in control group which might be considered as the normal percentage of damaged tubules. This percentage was found to be 6.19% and up to 7.83% in the treated groups I &II respectively.

Seminiferous tubules in treated animals were found to increase in size as measured by diameter, this was more obvious in group II (Fig3). The mean diameter of seminiferous tubules in animals of control group was found to 162.7± 0.487 m , this was considered as normal diameter, and it was found to be 170.2 ± 0.464 in group I and to reach 175.2 ± 1.596 in group II. The increase in diameter of the tubules showed significant difference at $P< 0.05$ between treated group I and control, and it becomes significant at $P<0.01$ between the treated group II and control group, as well as between the two treated groups I&II(Table 3).The interstitial tissue showed no proliferation and unaffected Leydig cells, the spermatogenesis was almost normal.

Table 3: Diameter of seminiferous tubules in micrometers (each figure is average of 15 randomly selected tubules), and the percentages of damaged tubules in the mice of the three groups.

| Animal No. | Control group | | Group I | | Group II | |
|---|---|---|---|---|---|---|
| | Average diameter | %of damaged tubule | Average diameter | %of damaged tubule | Average diameter | %of damaged tubule |
| 1 | 165.30 | 1.08 | 168.24 | 4.44 | 174.80 | 5.51 |
| 2 | 163.50 | 0.93 | 170.14 | 3.92 | 168.15 | 6.00 |
| 3 | 162.40 | 1.53 | 168.27 | 7.25 | 169.80 | 8.14 |
| 4 | 160.50 | 1.36 | 171.23 | 5.77 | 170.05 | 7.69 |
| 5 | 163.42 | 0.87 | 169.30 | 4.94 | 173.70 | 12.72 |
| 6 | 162.50 | 1.33 | 169.60 | 7.40 | 175.40 | 9.38 |
| 7 | 163.50 | 1.75 | 170.20 | 6.74 | 176.30 | 8.66 |
| 8 | 161.20 | 1.51 | 172.04 | 6.69 | 181.38 | 5.48 |
| 9 | 160.80 | 0.79 | 171.40 | 8.26 | 180.20 | 7.14 |
| 10 | 164.10 | 1.69 | 172.40 | 6.53 | 182.71 | 7.60 |
| Mean ±S.E. | 162.70 ±0.487 | 1.28 ±0.109 | 170.28* ±0.464 | 6.19 ±0.440 | 175.20** ±1.596 | 7.83 ±0.682 |

*Significant at $P<0.05$ **Significant at $P<0.01$ S.E.= Standard Error

# DISCUSSION

Results of this study were showed no mortality recorded during the period of experiment. Significant reduction of body weight related to feeding permethrin was agreement with similar findings of such weight reduction which was reported by Ishmael and Lichfield

(14) in mice fed the same insecticide at a level of 2500 mg/kg. More over signs of intoxication in the form of tremor and hyperactivity which were noticed in present work especially in group II also had been reported by Lichfield (12). Our data showed that permethrin induced slight increase in the weight of testes as well as in the testes -to-body weight ratio, this fact was reported earlier in the weight of certain organs such as liver, kidney (14,15) heart and spleen (15), all these observations were reported in male mice fed doses of permethrin ranging from 4000 to 10000 mg/kg.

In spite of the increase in the diameter of seminiferous tubules, the histological changes on testicular tissue were slight at the two doses used in this study, so majority of tubules were appeared unaffected with normal spermatogenic activity but in descending order, and the percentages of the damaged tubules were low, also Spencer and Brehance (16 ) did not report any statistically significant adverse effect in female pregnant rats given 4000 mg/kg permethrin in the daily diet. It is well known that the process of spermatogenesis is androgen-dependent, thus the reduction in androgen biosynthesis especially testosterone considerably affect spermatogenesis (17 ).Low level of testosterone

should lead to autolysis of seminiferous tubules and consequently to a reduction in the rate of spermatogenesis, these changes could be attributed to Leydig cells (18,19,20,21) and since administration of permethrin in the present work did not affect these cells i.e. locally produced testosterone by Leydig cells remained normal, therefore, least degree of tissue damage was noticed. Also it is well known fact that antispermatogenic insecticides have a direct affect on germinal epithelium and an indirect effect on Leydig cells thus on gonadotropin regulating hormone (22,23, 24).

It can be concluded that permethrin effect seems to be relatively low and does not affect the fertility of the mice. This low toxicity could partially be due to liability of the compound to rapid metabolism and elimination in a relatively short time (25,26). Metabolism of permethrin is carried out in mammals by induction of liver microsomal system through different pathways such as ester hydrolysis, oxidation and/or conjugation, consequently permethrin shows little or no affinity toward tissue accumulation (14,27,28).

Figure 1: Testis of control mice showing normal architecture, intact seminiferous tubules, spermatogenic cells and normal interstitial tissue. H & E. x 250.

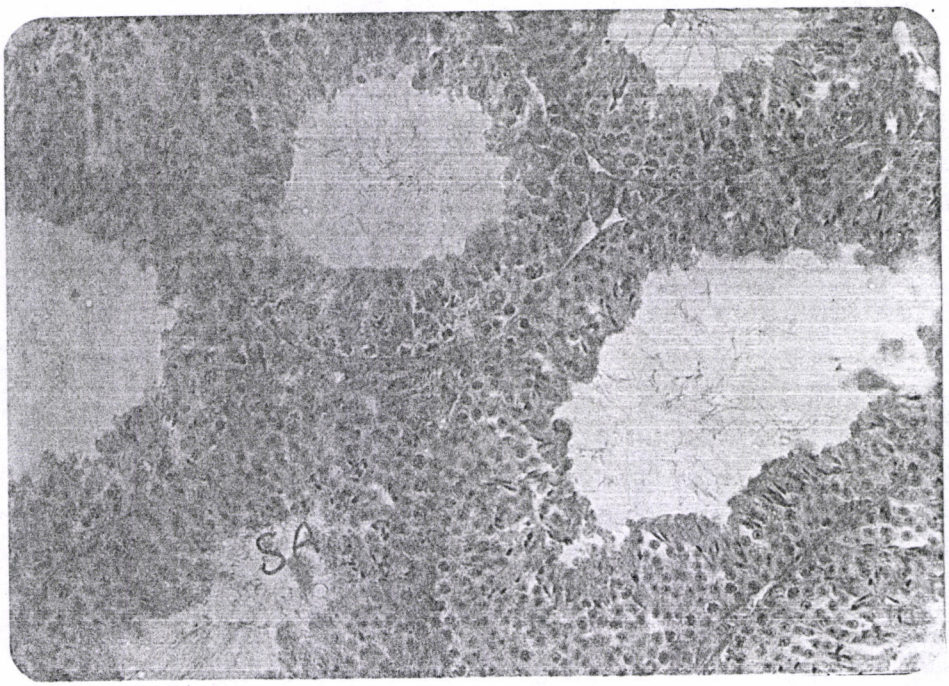


Figure 2: Testis of a mice fed 2000 mg/kg of permethrin showing slightly affected but intact semniferous tubules(SA), reduced spermatogenic activity and unaffected Leydig cells(U). H & E. x 250.


Figure 3: Testis of mice fed 3000 mg/kg of permethrin showing both intact and damaged seminiferous tubules(D), with descending order of spermatogenic activity and unaffected Leydig cells(U). H & E. x 250.

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# التغيرات النسجية المستحثة من المبيد الحشري البر مثرين في خصى الفئران البيض

خولة جاسم بلاش
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## الخلاصة

البر مثرين أحد المبيدات الحشرية الصناعية ذات السمية الواطئة للثدييات , يستعمل بصورة خاصة في الأغراض المنزلية. يهدف البحث إلى دراسة التأثيرات السمية لهذا المبيد على الخصى في الفئران وذلك بإعطائها وعن طريق الفم جرعتين مختلفتين هما 2000 ملغم/كغم و3000 ملغم/كغم من وزن الجسم ولمدة 30 يوم. عين تأثير المبيد من خلال حساب النسبة المئوية للنبيبات التالفة وقياس أقطار النبيبات ومعدل عملية نشوء النطف إضافة إلى التغير في وزن الجسم ونسبة وزن الخصية إلى وزن الجسم . أظهرت النتائج إن المبيد سبب انخفاض معنوي في وزن الجسم مع زيادة في نسبة وزن الخصية إلى وزن الجسم .كما أظهرت النبيبات المنوية زيادة معنوية في أقطارها مع درجات واطئة ومتفاوتة من التلف , أما عملية نشوء النطف فكانت طبيعية ولكنها منخفضة في حين لم تتأثر خلايا لديك ، نستنتج من هذا أن البر مثرين يمتلك تأثيرا" سميا" واطئا" على الخصى والتي يمكن اعتبارها غير مؤثرة على الخصوبة وخاصة في الجرع المستعملة .