TOXICITY TO MICE FED ON WHEAT FLOUR INFESTED WITH TENEBRIONID BEETLES

I.H. EL-KASHLAN[1], MADEHA ABDEL-HAMID[2], EGLAL HELAL[2], M.A. ABDEL-LATIF[2], LAILA EL-SHAZLY[3] AND MERVAT ALI-EL-DIN HAMZA[4]

1Plant Protection Research Institute, Agricultural Research Centre, Alexandria, Egypt.

2 Plant Protection Department, Faculty of Agriculture, Alexandria University.

3 Pathology Department, Medical Research Institute, Alexandria.

4 Pathology Department, faculty of Medicine, Alexandria University.

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Abstract

Feeding mice on infested wheat flour with three tenebroinid insect species, demonstrated strong toxic effect on the animals. Both liver and lungs of mice seem to be more sensitive than spleen. Morphological and histopathological studies made on these organs indicated obvious toxic symptoms probably due to the defensive secretion of benzoquinone compounds, discharged by tenebroinid insects. These compounds might have a carcinogenic effect on certain organs.

Keywords : Toxicity, mice, wheat flour, infected beetles, morphological, histopathological, symptoms, organs.

INTRODUCTION

The adults of family Tenebroidae (Coleoptera) possess a wide variety of glands releasing secretions which act as a defensive behaviour against competitor insects or its related biological agents.

The odoriferous glands in adult beetles consist of the glandular secreting cells and a large bifurcated reservoir for storing the secretion, evolved from a pair of invagination between the fifth and sixth abdominal sternites. These glands secrete a mixture of quinone compounds (Meinwald et al., 1966 and Eisner and Meinwald, 1966).

Research on the biosynthesis of arthoropod quinone was conducted by several authors (Meinwald et al., 1966 and Ladisch et al., 1967).

The biological activity of quinone particularly as carcinogens was discussed by several authors. A possible correlation between human malignancy and flour con-

taminated with quinone secreting by some tenebroid beetles was suggested by (Eisner and Meinwald, 1966; Ladisch et al., 1967 and Ganem, 1990). The relatively few available literature on feeding mice on flour infested with *Tribolium confusum, T.castaneum* and *Tenebrio molitor* revealed toxic symptoms . Chapman, 1926 was the first to mention that flour contaminated with secretions by some tenebroid beetles become red in colour. Several authors (Loconti et al, 1953; Schildknecht and Wels, 1960; Roth and Eisner, 1962; Tseng et al. 1971, Tschinkel, 1969 and 1972 and Markarian et al, 1978) studied the effect of secretion of some Tenebroids, namely *Tribolium castaneum, Eleodes longlcollis, Tenebrio molitor, Zophobad rugipes, Alphitolbius daperinus* and *Argoporis alutecea.* Mixture of quinone compounds and derivatives were identified. It contains, mainly 80-90% 2-ethyl-1,4- benzoquinone, 10-20% 2-methyl-1,4-benzoquinone and trace of 2-methoxy-1,4-benzoquinone, in addition to other compounds which may include unsaturated hydrocarbons.

Several investigators (Ladisch, 1953; Kishizawa, 1954; Yong and Kare, 1968; El-Mofty et al, 1980 and 1986 and Ganem, 1990) mentioned that bezoquinone compounds in the secretion of some beetles may have a carcinogenic effect on some organs of animals, mainly the liver, spleen and lung.

The aim of this study was to evaluate the toxic effect of feeding mice on flour infested with tenebroid beetles, and the extent of this effect on the liver, spleen and lungs.

MATERIALS AND METHODS

1 Experimental Animal

The Swiss albino mice *Mus musculus dometicus* L., have been considered and for a long time, the most widely used experimental animal. This extensive use is due to low breeding cost, small space requirement, tractability and omnivorous dietary needs. Also, short time spend of generation, large litters and the fact that the mice can be standardized.

Female and male mice each weighing an average 26-35 gm, were obtained from NAMRU-3 in Cairo and housed in metallic case. Thirty males and 30 females were divided into groups, each comprising 5 males and 5 females/cage. Five groups of mice were fed on flour infested with *Tribolium confusum. T.castaneum* and *Tene-*

brio molitor at different infestation levels. Another group was fed on clean wheat flour as control. The animal room was maintained at 22+1.5°C with a relative humidity of 50±10%. Water was provided ad libitum and changed at least every 1 to 2 days. Mice of about 8 weeks were used for treatments. Before starting the treatment animals were firstly fed on a prepared diet containing 100 gm clean wheat flour mixed with 125 ml warm water. One month later, when mice were about 12 weeks, flour infested with tenebroid beetles was offered 3 times per week, alternatively with wheat flour. (3 days on treated diet and 4 days on normal diet), until the death of the animal. Every three months, two females were provided with one male at 5 pm (mating likely occurs between 5 pm and 9 am). Each female was examined for the presence of a vaginal plug, it's presence was counted as Zero day of pregnancy. After mating, females were then reisolated alone.

2. Infestation of flour with tenebroinid insects

Wheat flour infested with the previous tenebroinid insects was prepared for mouse feeding experiments. Five rates of infestation were prepared using an approximate ratio 1:1:1 mixture of the respective species. The initial rates were 90 Insects/Kg, 70 Insects/Kg, 50 Insects/Kg, 30 Insects/Kg, 10 Insects/Kg and 0 Insects/Kg as a control. The prepared flour were stored for 6 months at 28°C ± 1 and 50 ± 10% R.H. for building up a considerable population of insects before being offered to the mice.

3. Morphological and histopathological studies

Periodically, every three months and for one year, one female and one male from each treatment were dissected for autopsy, that means a total of 48 animals for the whole experiment. Abnormalities in liver, spleen or lungs were described and photographed. These organs were then fixed in 10% formalin for histological and pathological studies . Histological methods adopted were done according to Addison, 1970. Pathological studies were carried out in collaboration with Faculty of medicine and High Institute of Public Health, Alexandria University.

RESULTS AND DISCUSSION

1 Toxicity of the mice

The results of feeding mice on flour infested with some tenebroid species indicated obvious toxic effect. From the data presented in Table 1 it was evident that a total mortality of 20% of mice occurred, but no mortalities observed at the lower

rates of infestation of 10 or 30 insects/Kg. Mortality occurred at the higher levels of infestation, and long exposure periods. It seems from the data in Table 1 that females were more sensitive than males as mentioned also by Ganem (1990).

Table 1. Number of dead mice females (F) and males (M) fed on infested wheat flour. (each treatments 5 females and 5 males mice).

Initial rate of infestation (Insect/Kg)*													
Duration of feeding (months)	Control		10		30		50		70		90		Total
	F	M	F	M	F	M	F	M	F	M	F	M	
3	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	1	1	0	2
9	0	0	0	0	0	0	0	1	0	1	0	0	2
12	0	0	0	0	0	0	2	0	2	0	1	1	6
Total	0	0	0	0	0	0	2	1	2	2	2	1	10

Tribolium confusum, T.castaneum, Tenebrio molitor, at the ratio 1:1:1.

Number of progeny per female, fed on infested flour shows that after 12 months, average percentage of reduction in progeny was about 30%, Table 2. The data in the same table also shows the number of dead and surviving litters per female at the tested levels of infestation. Mortality of born litters was obvious when mice were fed on flour contaminated with the highest levels of insect population.

Time factor expressed as a feeding period also plays considerable role in this respect. It is evident that treated females produced less number of progeny (alive and dead) especially for treatments of highest numbers of insects and long periods of exposure (1 dead and 3 alive for the treatment 90 insect/Kg and duration of feeding for 12 months).

2. Toxic morphological symptoms of some organs

Feeding mice on flour infested with tenebroinid insects was observed as changes in the morphology of certain organs, i.e. liver, spleen and lungs. Liver necrosis and nodules were observed, and the same symptoms were also observed in the spleen and lungs, Figs. 1,2. The changes were significantly correlated with the density of insects in flour and the duration of feeding. It was evident that both the liver and the lungs were more sensitive to the chemicals than the spleen.

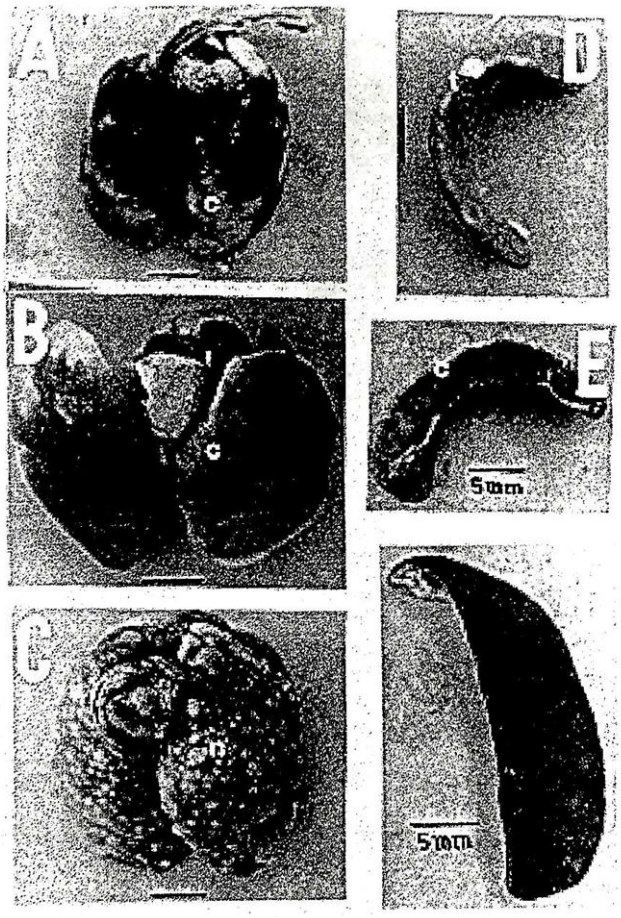

Fig. 1. Lung (A.B.C) and spleen (D.E.F) of mouse (age of 50 weeks) (initial infestation 90 insect/1 kg.) fed on treated wheat flour for 12 months.

(c) area of necrosis

(n) nodules

(t) tumor.

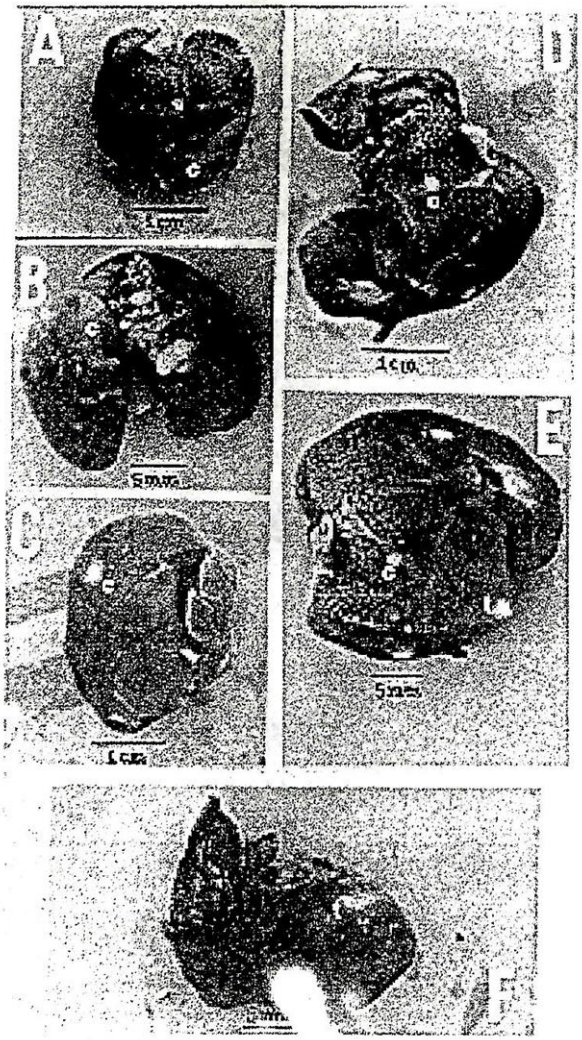

Fig. 2. liver of mouse (age of 50 weeks) (initial infestation 90 insect/1 kg) fed on treated wheat flour for 12 months.

(c) area of necrosis

(t) tumor.

Table 2. Number of dead mice (D) and live (L) progeny for one mated female mouse fed on wheat infested with three Tenebroid sp.

Duration of feeding (months)	Initial rate of infestation (Insect/Kg)*																Average treatment
	Control		10		30		50		70		90		Total				
	D	L	D	L	D	L	D	L	D	L	D	L	D	L			
3	-	9	-	9	-	8	-	9	-	8	1	5	1	39	8.0		
6	-	7	-	7	-	6	-	7	-	6	-	3	-	29	5.8		
9	-	9	-	10	-	8	2	7	1	3	2	3	5	31	7.2		
12	-	10	-	7	-	9	2	9	2	-	1	3	5	27	6.4		
Total	-	35	-	33	-	31	4	31	3	17	4	14	11	126			
Av.no.of D and L	8.7		8.2		7.7		8.7		5.0		4.5						

D = Dead litters

L = Live litters accrod. to WHO list

**Tribolium confusum, T.castaneum, Tenebrio* molitor, at the ratio 1:1:1.

Table 3 demonstrates the changes as number of mice that have abnormal target organs due to the treatments. Rate of toxicity revealed that 36 mice (20 females and 16 males) out of the tested 60 mice (60%), exhibited abnormal changes in their organs. Also, 40 of these organs (33%) out of 120 investigated organs were badly morphologically changed.

As reported by Roth and Eisner, 1962 and El-Mofty et al., 1980 and Ganem, 1990 it seems that most of the toxic symptoms were probably due to the effect induced by the secretion of quinone by tenebroinid insects. The bioactivity of these chemicals may be due to either the irritating effect of quinone, or their metabolises on the tissues.

During a year of observation, the tested mice demonstrated variable toxic symptoms in their target organs, i.e. nodules of different sizes and numbers, necrosis surrounded by inflamentory cells. However, it could be concluded that the experiments failed to demonstrate a consistent pattern of effect and a consequence degree of toxic symptoms. This was mainly due to the available numbers of tested mice and to the nature of biological experiments.

Generally, it could be mentioned, that factors under investigation i.e. duration

Table 3. Effect of feeding mice on flour infested with stored product insects on mice organs based on morphological changes.

No. of months	Sex	Initial rate of infestation (Insect/Kg)*																		Total
		Control			10			30			50			70			90			
		L	Sp	Lu	L	Sp	Lu	L	Sp	Lu	L	Sp	Lu	L	Sp	Lu	L	Sp	Lu	
3	F	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	3
	M	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	3
6	F	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	4
	M	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2
9	F	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	3
	M	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	3
12	F	0	0	0	1	1	1	1	0	1	1	0	1	0	0	1	1	0	1	10
	M	0	0	0	1	1	1	0	0	0	1	0	1	0	1	1	1	0	0	8
Total		0	0	0	2	3	3	2	1	2	4	0	3	2	3	3	5	1	2	36

L= Liver SP = Spleen Lu = Lung

F = Female M = Male

of mice feeding and number of insects/Kg flour, play a considerable role in inducing toxic effects.

Severe and extensive damage to the investigated organs were observed when mice were exposed to heavily infested food for long periods.

3. Histological and pathological changes

Hematoxylin and eosin stained paraffin sections were carried out in the liver, spleen and lung of mice exposed to infested food by tenebroind beetles. Specimens of liver indicated that damage to the tissues started with a slight change as necrosis in the portal tract where blood vessels were congested, surrounded by inflammatory cells and degenerative changes in cytoplasm. Also, deposition of brownish material called hemosiderin (Addison, 1970 and El-Shazly et al. 1987) was observed. Then multiple nodules and necrosis, Figs. 3. to 8, were observed.

In late stages i.e. 12 months, architecture disturbance of liver tissue was observed, by multiple nodules which cause necrotic tissue and cellular debris surrounded by band of fibrosis. A large necrotic eosinophilic focus observed. Central veins are widely apart more than normal, like in hepatoma, Ladisch et al., 1967 and Markarian et al., 1978. More fibrosis was observed and blood vessels were more congested. Liver cells in some areas showed variations in size of both cytoplasm and nuclei. Giant cells and fibrosis were seen in abundance in portal tracts. Hyperbasophilic nodules and a typical foci of liver cells were frequently met with hepatoma or may be hepatocarcinoma, were suspected as reported by Miller et al., 1978 and El-Shazly et al., 1987.

Concerning the spleen, the pathological changes could be identified as areas of necrosis surrounded by inflammatory cells and blood vessel congestion.

The investigated lungs of treated mice in the early stages exhibited group of inflammatory cells. Also, solid and collapsed tissue was observed, and blood vessel congestion. Later on, massive area of necrosis was noticed surrounded by inflammatory cells, congestion and embolus was found in bronchus. Severe symptoms were noticed, as collapse lungs tissue and collection of inflammatory cells. Alveolus were not seen, diagnosed as atelectatic. Compancetory dilation of alveolus untreated was diagnosed as emphysema as reported by El-Shazly et al., 1987.

Thus, it could mentioned that feeding mice on wheat flour infested with tene-

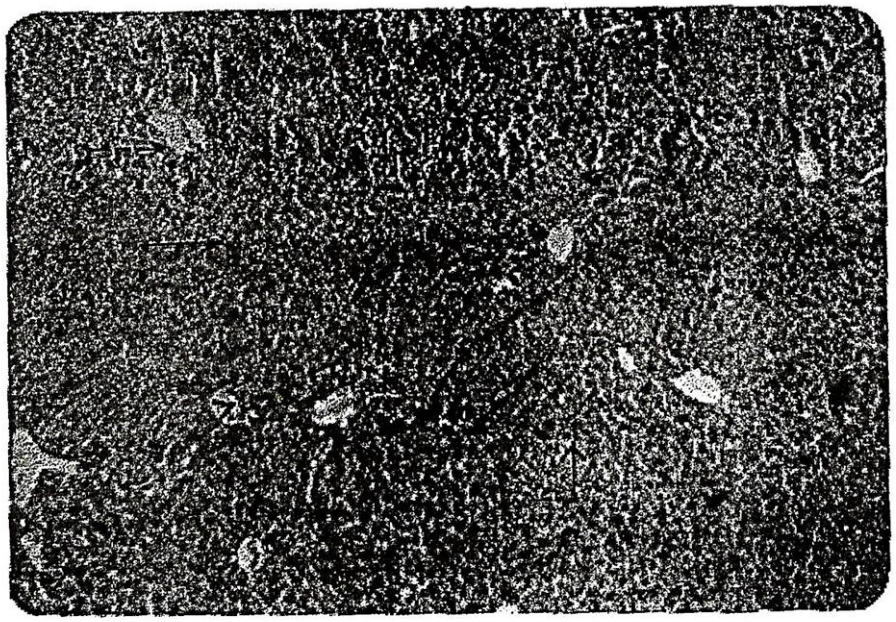

Fig. 3. liver tissue section of normal mouse (x 75)
Hepatacyte (➡)
Hepatic cord (--➡)
Lymhocyte hepatic sinusoids (<).

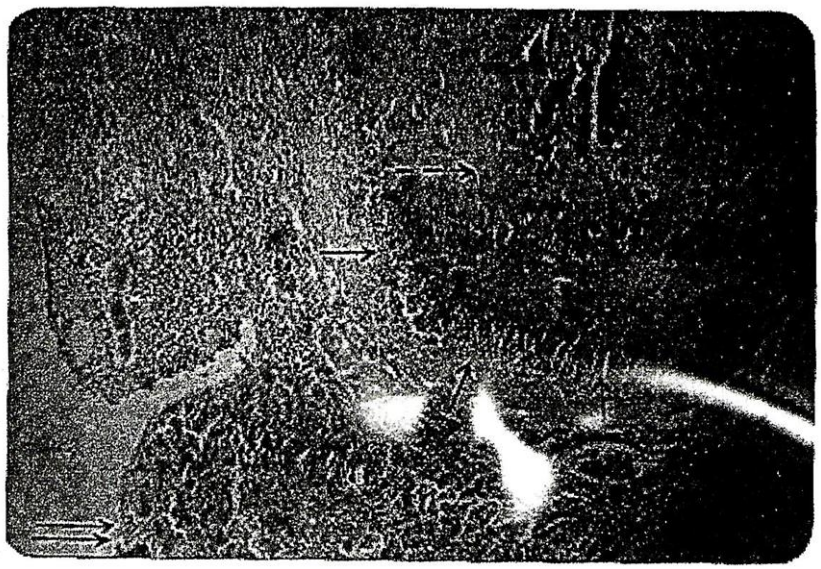

Fig. 4. liver tissue section of mouse fed on infested wheat flour by tenebroinid beetles for 12 months.
Extensive area of necrosis (➡)
No special architecture (--➡)

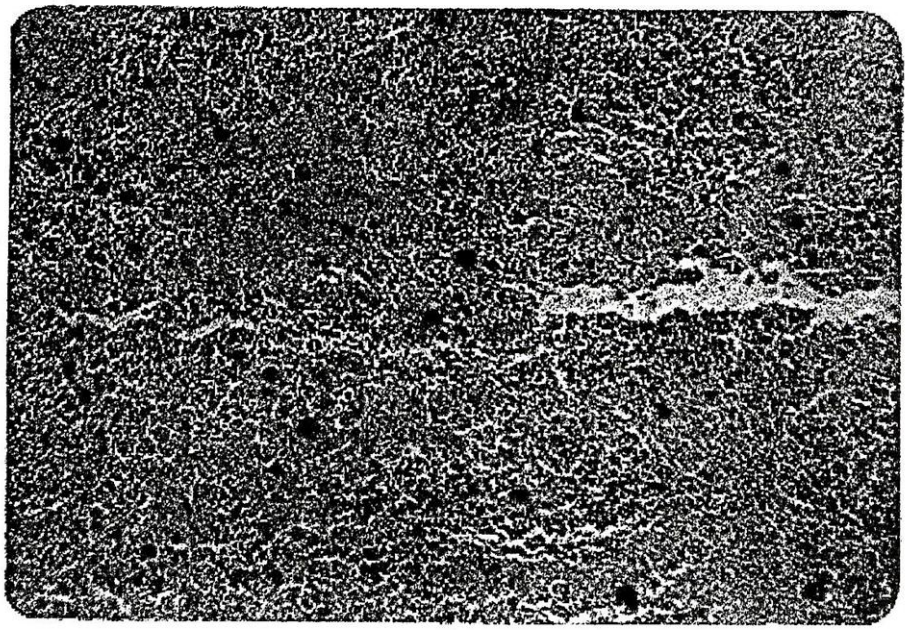

Fig. 5. Spleen tissue section of normal mouse (x 75)

Fig. 6. Spleen tissue section of mouse fed on infested wheat flour by tenebroinid beetles for 12 months

Necrotic area (➡)

Blood vessel congested (--➡) (x 75)

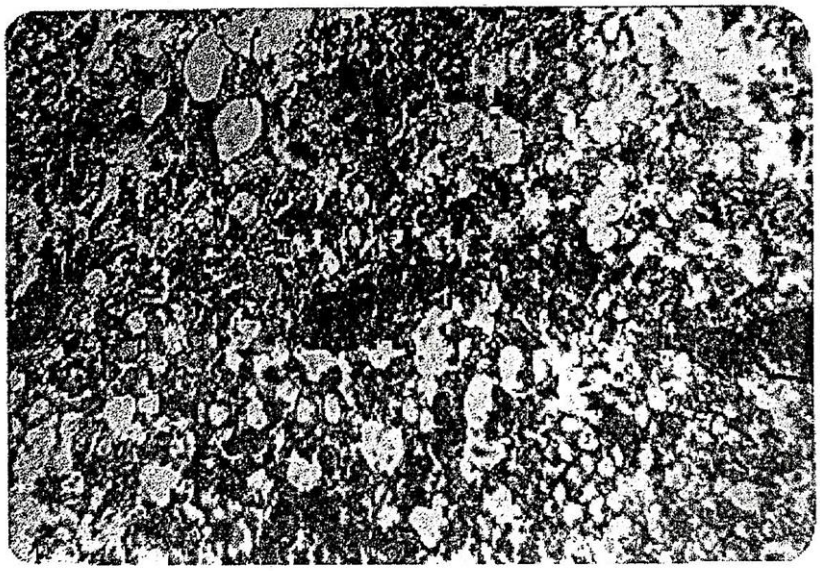

Fig. 7. Lung tissue section of normal mouse (x 75).

Fig. 8. Lung tissue section of mouse fed for 3 months on infested wheat flower by tenebroind beetles solid and collapse lung tissue (➡)
Collection of inflammatory celis (--➡) (x 75)

broinid beetles may induce tumor-like characteristics in vital organs as the liver and lungs. The damage done to these organs and others could probably be due to the toxicity of secreted chemicals from the defensive glands.

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التأثير السام لتغذية الفئران على دقيق القمح المصاب ببعض خنافس عائلة Fam: Tenebrionidae

ابراهيم هانى القشلان١ ، مديحة عبد الحميد٢ ، اجلال هلال٢ ،
محمد عباس عبد اللطيف٢ ، ليلى الشاذلى ٣ ، مرفت حمزة ٤

١ معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الاسكندرية .

٢ قسم الحشرات الاقتصادية – كلية الزراعة – جامعة الاسكندرية .

٣ قسم الباثولوجى – معهد البحوث الطبية – الاسكندرية.

٤ قسم الباثولوجى – كلية الطب – جامعة الاسكندرية.

أظهرت النتائج بوضوح أن تغذية الفئران التى استخدمت كحيوانات تجارب بيولوجيه على الدقيق المصاب ببعض حشرات عائلة Fam : Tenebrionidae (رتبة غمدية الاجنحة Coleoptera) له تاثير سام بأشكال مختلفة. فقد أظهرت الدراسة المورفولوجية أن الكبد والرئة أكثر الأعضاء حساسية وتأثرا عن الطحال.

وبالدراسة الهيستوباثولوجية ظهر التأثير السام بوضوح على الكبد والرئة والطحال نتيجة لافراز الغدد الدفاعية لحشرات هذه العائلة لمادة البنزوكينون ومشتقاتها فى الدقيق المصاب.

وقد وجد أيضا من هذه الدراسة انه من المحتمل ان مادة البنزوكينون لها تأثير سرطانى على الاعضاء الداخلية ، كما هو واضح من الصور المرفقة.