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THE HISTOPATHOLOGICAL EFFECTS OF WARFARIN AND CHLOROPHACINONE ANTICOAGULANT RODENTICIDES ON MALE ALBINO RATS.

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ABSTRACT

The histopathological effects of warfarin and chlorophacinone anticoagulant rodenticides were studied on liver, kidney and testis of male albino rats *Rattus norvegicus* after administration of sub – lethal doses ($^1/_{10}$ and ¼ LD_{50}) after 15 days post – treatment. The microscopical examination showed that there were injuries in these organs depending on the dose level. In liver, both tested compounds induced dilation of central and portal vein, congestion with lymphocytic infiltration and necrosis. In kidney , the two tested compounds occurred renal cloudy swelling, congestion, lymphocytic infiltration, focal areas of haemorrhage in the internal tubular connective tissues and necrosis. Also, the effect of the two compounds on the testis showed that there were thickness basement membrane and lymphocytic infiltration of seminiferous tubules.

INTRODUCTION

There are several anticoagulant rodenticides in use today which have a similar chemical structure to warfarin and others which are not related chemically,but have similar physiological action. In Egypt, the anticoagulant rodenticides have been used on a large scale to control rodents either in agriculture or for public health purposes. On the other hand, some anticoagulas as warforin have been used in different m edical p urposes a s drugs. It was found to has a number of potential harmful effects as histological changes in tissues of some orgons (Haram *et al.*,1993). It was important to fined another effects in the using of anticoagulant rodenticides.

In this work, an attempt had been conducted to study the histopathological effects of warfarin and chlorophacinone anticoagulant rodenticides on the different organs of albino rat.

MATERIALS AND METHODS

1- Rodenticides Used :-

Two a nticoagulant r odenticides o btained f rom K z pesticides Co., Egypt were used; these are:

- Warfarin (98%) : 3- ((- acetonly benzyl)4- hydroxy coumarin.
- Chlorophacinone (90%): 2-[2- (4 – chlorophenyl)- 2 – phenyl acetyl] indan – 1,3 – dione.

2- Tested Animals :-

The adult individuals of male albino rats *Rattus norvegicus* were used. Animals were caged individually with diet and water supplied *ad libitum.* The unhealthy animals were excluded. Animals were weighed and given a reference number for each one.

3 - Procedures :-

3 – 1-LD_{50} Determination :-

Serial doses of warfarin and chlorophacinone active ingredient calculated as mg/kg body weight, were prepared and suspended in corn oil. Four animals were used for each dose and administered by oral intubation. Animals were fasted for 12h before the treatment. A parallel control test was conducted using plain corn oil. Mortality percentages were recorded up to 28 days after the treatment. LD_{50} values were calculated using the probit transformation table as designed by weil (1952)and simplified by Horn(1956).

3-2- The Histopathological Studies:-

Animals were divided into groups (each of 5 rats), four groups for the treatment and another one as a control. Animals were fasted for 12h before the treatment, then orally intubated with sub-lethal doses, $^{1}/_{10}$ and ¼ LD_{50} of warfarin and chlorophacinone (one dose for each group). Doses were prepared and suspended in corn oil. Animals were decapted after 15 days from the treatment and the specimens (organs) were fixed in alcoholic Bouin's solution for about 24h, then washed. Few drops of Lithium carbonate were used to wash out the picric acid from the material. Samples were then dehydrated in standard alcoholic series and cleared in xylol before embedding in paraffin wax. Samples were then serially sectioned at the thickness of 2-3 microns and stained according to the techniqe of Conn and Darrow (1960), using delefields haematoxylin and eosin which was found satisfactory for the purpose of the present study. The tissues sections were then examined under light microscopy and photographed for histological evaluation.

RESULTS AND DISCUSSION

1- Acute Oral Toxicity (LD_{50}) :-

The LD_{50} of warfarin and chlorophacinone anticoagulants was determined in order to choose the suitable dose for further experiments. Data in Table (1) showed that mortality percentages increased with increasing dose whereas doses of warfarin 8.4 , 14.5 , 36.0 and 52.0 mg/kg caused 25, 50, 75 and 100 % mortality, respectively. On the other hand chlorophacinone anticoagulant was more effective than warfarin as the same mortality percentages were occurred with doses of 2.19 , 3.15 , 4.54 and 6.53 mg / kg , consecutively.It is cleared that male albino rats are more susceptible to chlorophacinone than warfarin as the calculated LD_{50} was 3.15 mg/kg comparing with 14.50 mg/kg for warfarin. These values agree approximatly with values that estimated by kandil *et al.* (1995) and El – Deeb *et al.* (2002).

Table (1) : Determination of LD50 of warfarin and chlorophacinone to male albino rats.

Compound	Dose	% Mortality	LD50 mg / kg
Warfarin	8.4	25	14.5
	14.5	50	
	36.0	75	
	52.0	100	
Chlorophacinone	2.19	25	3.15
	3.15	50	
	4.54	75	
	6.53	100	

2 - The Histopathological Effects :-

The histopathological effects of warfarin and chlorophacinone anticoagulant rodenticides on some internal organs (liver ,kidney and testis) of albino rats, *Rattus norvegicus* after oral administration of sub – lethal doses ($^1/_{10}$ and ¼ LD_{50}) were studied at 15 days post – treatment comparing with untreated animals and illustreated in Figs.(1-15).

2-1- Liver :-

The histopathological effect of chlorophacinone in the liver was illustrated in Fig (2-3). Fig (2) showed the microscopical examination of liver tissus revealed that karyopyknosis of nuclei of hepatic cells and dilation of central and portal vein at dose of $^1/_{10}$ LD_{50}. Also Fig . (3) show that dilation of central and portal vein with lymphocytic infiltration and necrosis at dose of ¼ LD_{50} comparing with control (Fig . 1).

Regarding warfarin treatment, Fig.(4) illustrated histopathological changes in liver at dose $^1/_{10}$ LD_{50} . It is clear that, hepatic changes of dilation of central and portal vein. Also, Fig . (5) indicated that, there were hepatic changes of congestion with necrosis and dilation of central and portal vein at dose ¼ L D_{50}. A similar investigation was carried out on the liver of some mammals species by Hamed (1979) who confirmed our results as he mentioned that organophosphorus compounds induced dilation and engorgation in central veins of rats, sinusoids and portal veins, in addition to lymphocytes and macrophages infiltration and fibroplastic proliferation in portal area associated with necrobiotic changes in the hepatocytes. Also, our findings agree with El – Mansoury (1983), Mahran (1990) , Fares (1996) and Zaghloul & El –Daly (1996).

2-2 - Kidney :-

The histopathological effects of chlorophacinone anticoagulant on kidney of albino rats illustraed in Figs. (7 & 8) in comparison with control (Fig. 6). At the dose of $^1/_{10}$ LD_{50} , microscopically, renal cloudy swelling and necrosis had been seen (Fig . 7). Also, at the dose of ¼ LD_{50} , it showed congestion and lymphocytic infiltration had been also seen as shown in Fig. (8). On the other side, the treatment with $^1/_{10}$ LD_{50} of warfarin revealed that renal lesions comprising congestion, lymphocytic infiltration and focal area of haemorrhage internal tubular connective tissues had been seen (Fig .9).

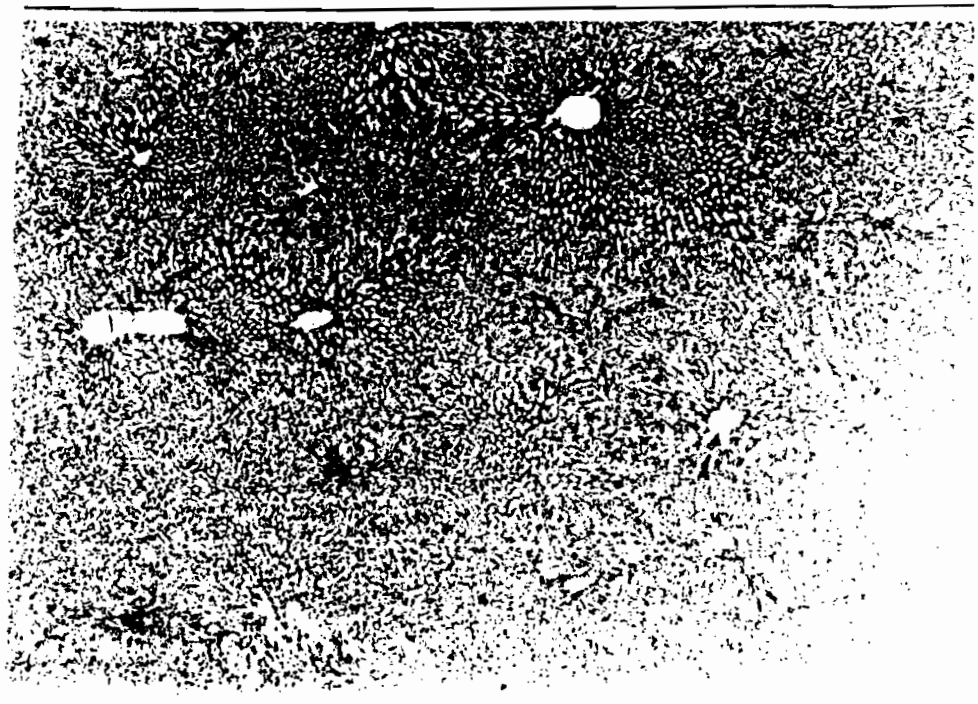

Fig. (1) : Liver tissue of untreated albino rat (control).

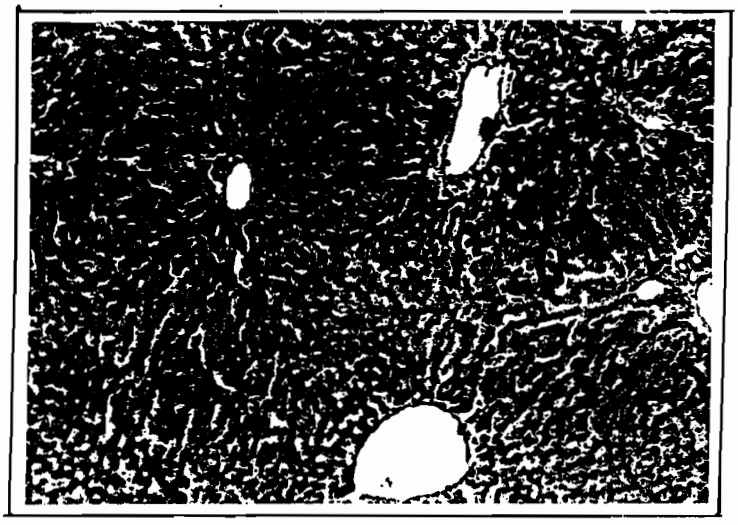

Fig. (2) : Liver tissue of albino rat treated with 1/10 LD_{50} of chlorophacinone.

Fig. (3) : Liver tissue of albino rat treated with 1/4 LD_{50} of chlorophacinone.

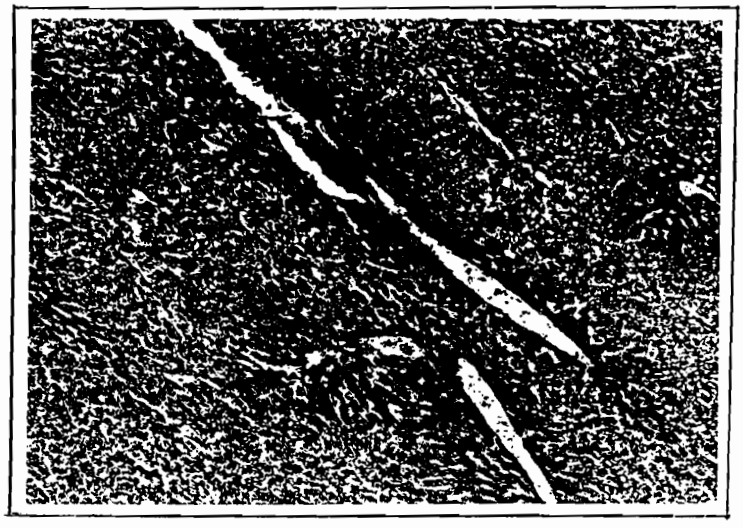

Fig. (4) : Liver tissue of albino rat treated with 1/10 LD_{50} of warfarin.

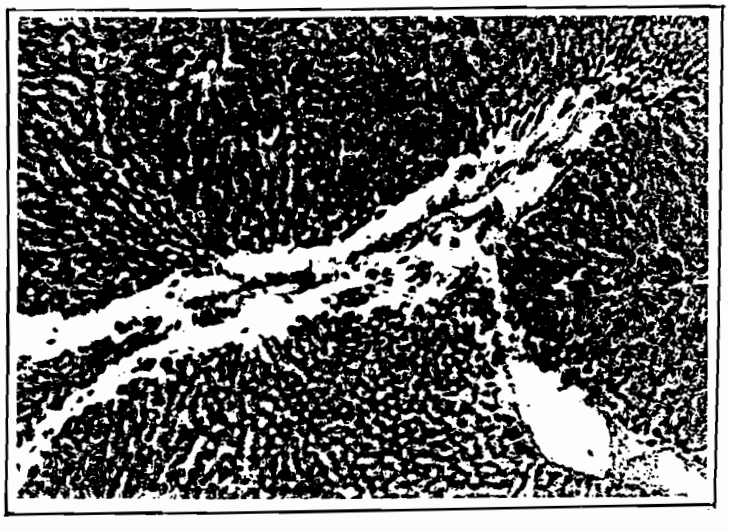

Fig. (5) : Liver tissue of albino rat treated with 1/4 LD_{50} of warfarin.

Fig. (6) : Kidney tissue of untreated albino rat (control).

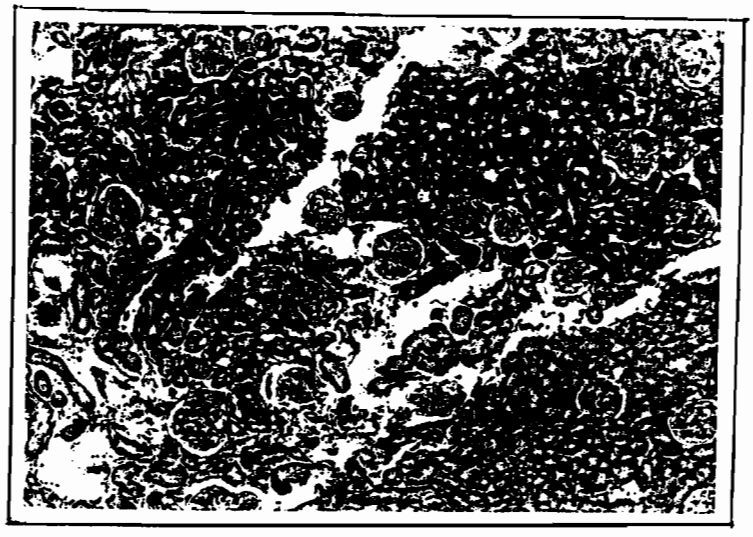

Fig. (7) : Kidney tissue of albino rat treated with 1/10 LD_{50} of chlorophacinone.

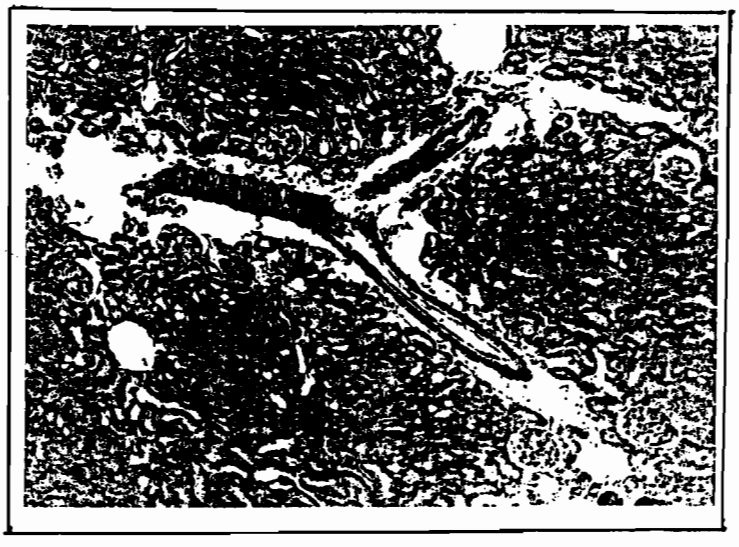

Fig. (8) : Kidney tissue of albino rat treated with 1/4 LD_{50} of chlorophacinone.

Fig. (9) : Kidney tissue of albino rat treated with 1/10 LD_{50} of warfarin.

Fig. (10) : Kidney tissue of albino rat treated with 1/4 LD_{50} of warfarin.

Fig. (11) : Testis tissue of untreated albino rat (control).

Fig. (12) : Testis tissue of albino rat treated with 1/10 LD_{50} of chlorophacinone.

Fig. (13) : Testis tissue of albino rat treated with 1/4 LD_{50} of chlorophacinone.

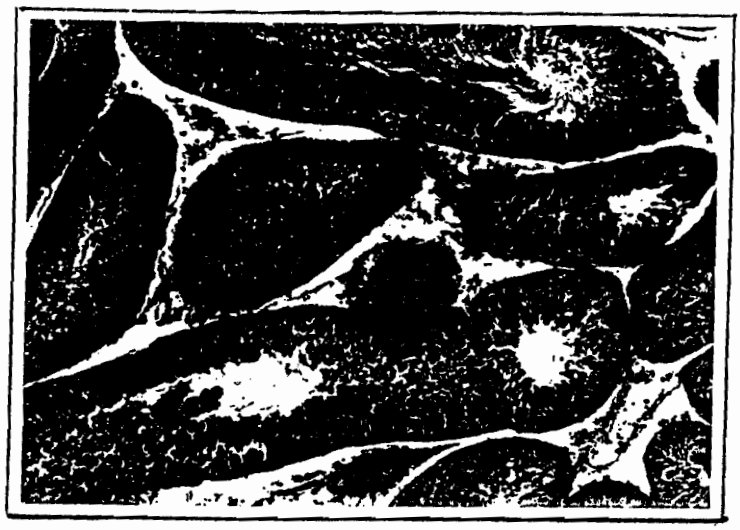

Fig. (14) : Testis tissue of albino rat treated with 1/10 LD_{50} of warfarin.

Fig. (15) : Testis tissue of albino rat treated with 1/4 LD_{50} of warfarin.

At the same time , ¼ LD_{50} of warfarin showed renal cloudy swelling and necrosis (Fig .10). These findings are in agreement with Hamed (1979) who noticed the presence of albuminous material in the kidney tubules of rats at $^1/_5$ LD_{50} of racoumin anticoagulant. Similar results were observed by El-sabbagh (1992).

2-3- Testies :-

The h istopathological effects of chlorophacinone rodenticide on the testies of albino rats were illustrated in Figs.(12&13) comparing with untreated (Fig.11). At doses of $^1/_{10}$ and ¼ LD_{50}, fibrosis with lymphocytic infilteration of seminiferous tubules was seen. On the other hand warfarin at dose of $^1/_{10}$ LD_{50} , microscopically, it showed thichening basement membrane had been seen (Fig .14). While at dose of ¼ LD_{50} , the microscopical examination showed thickening basement membrane and lymphocytic infiltration of seminiferous tubules was detected (Fig .15). These results are in harmony with those obtained by Baronia & Sahai (1993) and Saleh (1996).

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التأثيرات الهستوباثولوجية لمبيدي القوارض المضادة للتجلط الوارفارين والكلوروفاسينون علي ذكور الجرذ الألبينو

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية – دقي – جيزة

تم دراسة التأثيرات الهستوباثولوجية لمبيدي القوارض المضادة للتجلط الوارفارين والكلوروفاسينون في الكبد والكلي والخصية علي ذكور الجرذ الألبينو بعد ١٥ يوم من المعاملة عن طريق الفم بجرعات تحت مميتة مقدارها ١/١٠ ، ١/٤ من قيمة الجرعة المميتة للنصف LD_{50} . وأظهرت الدراسة من خلال الفحص السيكروسكوبى أن تأثير المعاملة بهذين المركبين على هذه الأعضاء كان واضحا و يعتمد على مستوى الجرعة المعطاة.

بالنسبةللكبد، أوضحت النتائج أن كلا المركبين تسبب فى حدوث أتساع و تهتك فى جدر الأوعية الدموية و الوريد المركزى و البابى مع حدوث أحتقان وأرتشاحات ليمفاوية ونخر في الخلايا . وفي حالة الكلي أحدثت المعاملة بكلا المبيدين تضخم كثيف وأحتقان مع إرتشاح ليمفاوي و ظهور بؤر نزيفية في الأنسجة الضامة للأنابيب الداخلية مع حدوث نخر واضح في الخلايا . أما بالنسبة لتأثير المعاملة بكلا المركبين علي الخصية ، فقد أظهرت المعاملة حدوث تغلظ في سمك الجدار القاعدي وكذلك حدوث إرتشاح ليمفاوي في الأنابيب المنوية .