

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

El-Deeb, H.I.; W.M. Gabr; Fatma K. Khidr and E.A. El-Essely
Plant Protection Research Institute, Agr. Res. Center, Dokki – Giza.

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 $1/4$ LD₅₀) 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 anticoagulants as warfarin have been used in different medical purposes as drugs. It was found to have a number of potential harmful effects as histological changes in tissues of some organs (Haram *et al.*, 1993). It was important to find 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 anticoagulant rodenticides obtained from Kz pesticides Co., Egypt were used; these are:

- Warfarin (98%) : 3- (4 - acetylbenzyl)-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-LD₅₀ 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. LD₅₀ 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, $\frac{1}{10}$ and $\frac{1}{4}$ LD₅₀ of warfarin and chlorophacinone (one dose for each group). Doses were prepared and suspended in corn oil. Animals were decapitated 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 technique of Conn and Darrow (1960), using deLeffields 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 (LD₅₀) :-

The LD₅₀ 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 LD₅₀ was 3.15 mg/kg comparing with 14.50 mg/kg for warfarin. These values agree approximately 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 $1/4$ LD₅₀) were studied at 15 days post – treatment comparing with untreated animals and illustrated 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 tissue revealed that karyopyknosis of nuclei of hepatic cells and dilation of central and portal vein at dose of $1/10$ LD₅₀. Also Fig . (3) show that dilation of central and portal vein with lymphocytic infiltration and necrosis at dose of $1/4$ LD₅₀ comparing with control (Fig . 1).

Regarding warfarin treatment, Fig.(4) illustrated histopathological changes in liver at dose $1/10$ LD₅₀ . 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 $1/4$ LD₅₀. 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 illustrated in Figs. (7 & 8) in comparison with control (Fig. 6). At the dose of $1/10$ LD₅₀ , microscopically, renal cloudy swelling and necrosis had been seen (Fig . 7). Also, at the dose of $1/4$ LD₅₀ , it showed congestion and lymphocytic infiltration had been also seen as shown in Fig. (8). On the other side, the treatment with $1/10$ LD₅₀ of warfarin revealed that renal lesions comprising congestion, lymphocytic infiltration and focal area of haemorrhage internal tubular connective tissues had been seen (Fig .9).

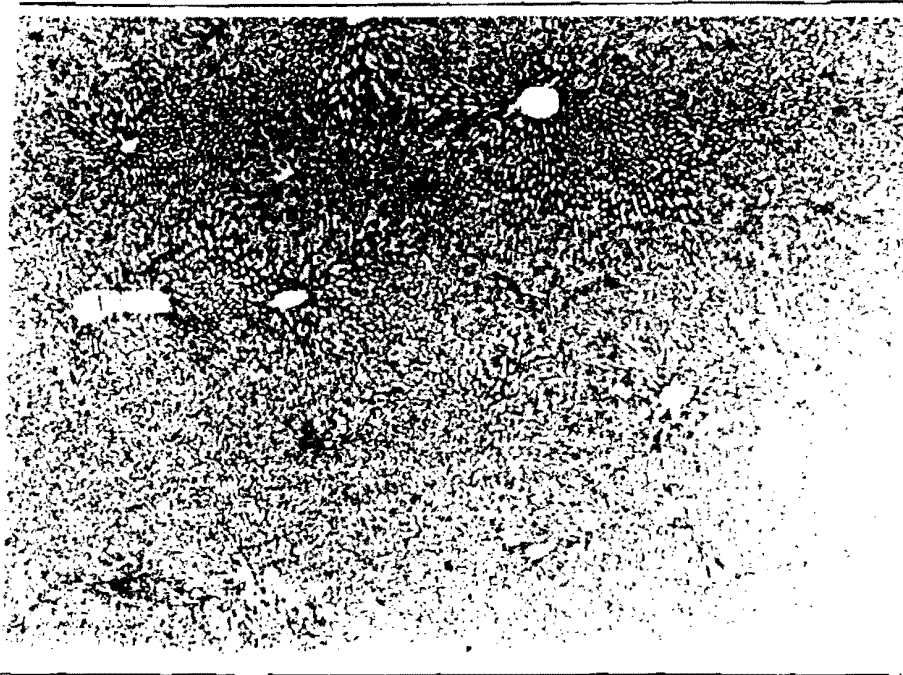


Fig. (1) : Liver tissue of untreated albino rat (control).

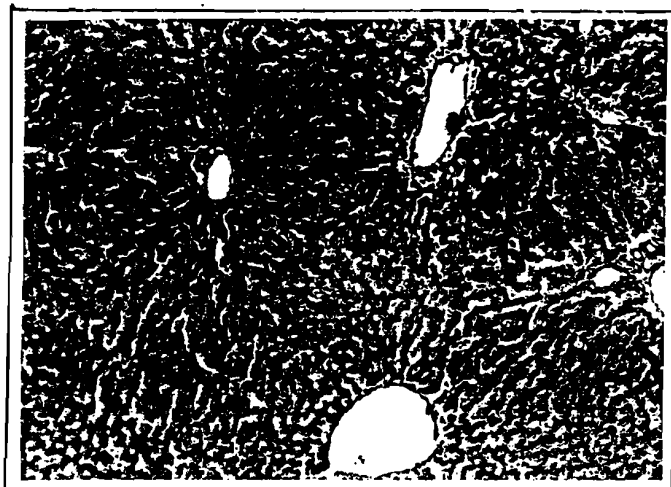


Fig. (2) : Liver tissue of albino rat treated with 1/10 LD₅₀ of chlorophacinone.

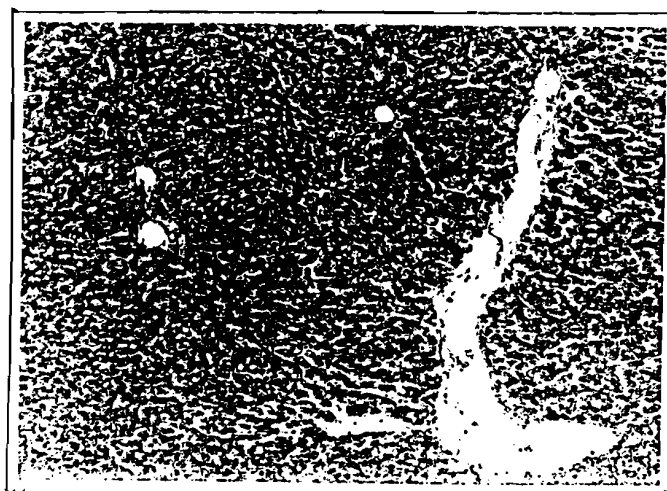


Fig. (3) : Liver tissue of albino rat treated with 1/4 LD₅₀ of chlorophacinone.

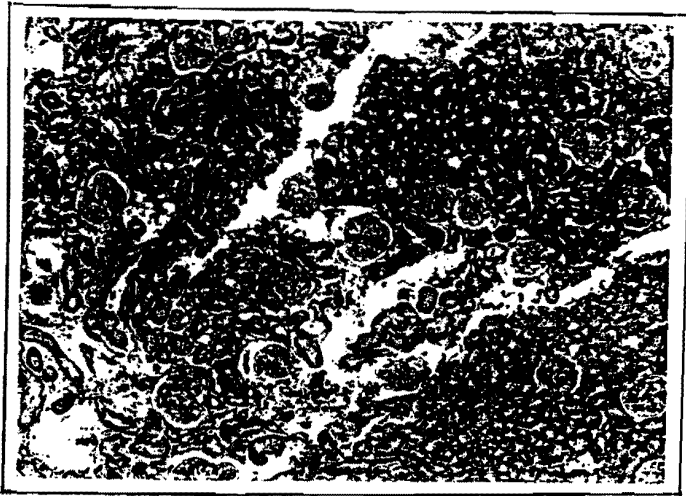


Fig. (7) : Kidney tissue of albino rat treated with 1/10 LD₅₀ of chlorophacinone.

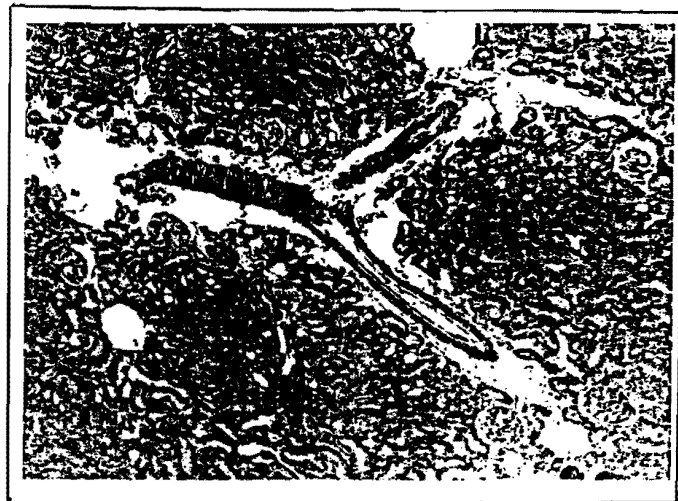


Fig. (8) : Kidney tissue of albino rat treated with 1/4 LD₅₀ of chlorophacinone.



Fig. (9) : Kidney tissue of albino rat treated with 1/10 LD₅₀ of warfarin.



Fig. (10) : Kidney tissue of albino rat treated with 1/4 LD₅₀ of warfarin.



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



Fig. (12) : Testis tissue of albino rat treated with 1/10 LD₅₀ of chlorophacinone.



Fig. (13) : Testis tissue of albino rat treated with 1/4 LD₅₀ of chlorophacinone.

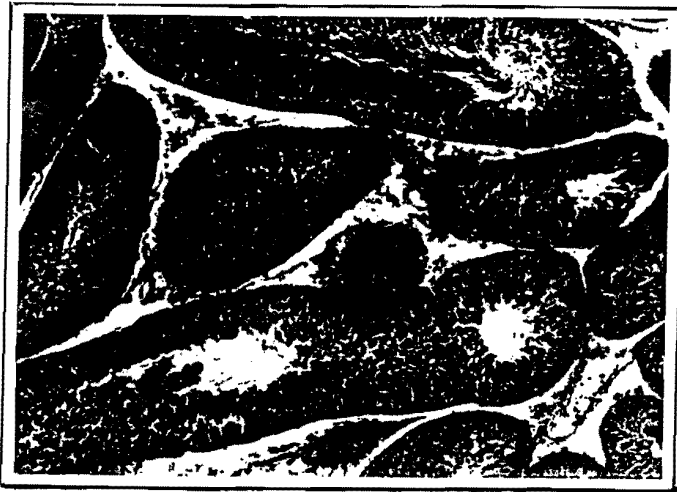


Fig. (14) : Testis tissue of albino rat treated with 1/10 LD₅₀ of warfarin.



Fig. (15) : Testis tissue of albino rat treated with 1/4 LD₅₀ of warfarin.

At the same time, $\frac{1}{4}$ LD₅₀ 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 $\frac{1}{5}$ LD₅₀ of racoumin anticoagulant. Similar results were observed by El-sabbagh (1992).

2-3- Testies :-

The histopathological effects of chlorophacinone rodenticide on the testies of albino rats were illustrated in Figs.(12&13) comparing with untreated (Fig.11). At doses of $\frac{1}{10}$ and $\frac{1}{4}$ LD₅₀, fibrosis with lymphocytic infiltration of seminiferous tubules was seen. On the other hand warfarin at dose of $\frac{1}{10}$ LD₅₀, microscopically, it showed thickening basement membrane had been seen (Fig. 14). While at dose of $\frac{1}{4}$ LD₅₀, 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).

REFERENCES

- Baronia, A.K. and Y.N. Sahai (1993). DDT induced changes in the testis of albino rat, a histopathological study. *J. Environ. Biol.*, 14, 2, 153 – 156.
- Conn, H.J. and M.A. Darrow (1960). Staining procedures used by the biological stain commission, 2, Geneva N.Y. Biotech. Publication.
- El-Deeb, H.I., M.H. Khalifa; W.M. Gabr and E.A. El-Essely (2002). Evaluation of two anticoagulant rodenticides as chemosterilants in female albino rats. *J. Union Arab Biol. Cairo*, 17 (A), Zoology, 231-240, Sept. 2002.
- El-Mansoury, H.A. (1983). Toxicological study on the hazards occurring as a result of application of herbicide " Gesaprim " M.Sc. Thesis, Fac. Vet. Med., Cairo Univ.
- El-Sabbagh, H.S. (1992). The role played by sex in modification of toxic action of some pesticides. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
- Fares, N.H. (1996). Histological and fine structural changes in the kidney cortex of mice treated by carbamate insecticide sevin. *J. Egypt. Ger. Soc. Zool.*, 21 (c), 53-85.
- Hamed, R.I. (1979). Some toxicological studies on racoumin in dog. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
- Haram, K.H.; T.T. Hrafnkell and A. Lage (1993). Anticoagulant activity sulfatide and its antithrombotic effect in rabbit. *J. Biochem.*, 113, 6, 781-785.
- Horn, H.J. (1956). Simplified LD₅₀ for (or ED₅₀) calculations. *Biometrics*, 12, 311-322.
- Kandil, M.A.; M.A. El-sayed and H.K. said (1995). Biochemical fingerprints of the commensal rodents in Egypt and their susceptibility to warfarin. 3rd Cong. Toxicol. Dev. Count., Cario, 19-23 Nov. III, 27 – 38.
- Mahran, H.N. (1990). Histological and histochemical studies on the effects of the rodenticide brodifacoum on the liver and small intestine of the white rat. M.Sc. Fac. Science, Menofia Univ.

- Saleh, A.T. (1996). Effect of carbamate insecticide, Iannate, on the spermatogenesis in mice. J. Egypt. Ger. Soc. Zool., 20 (c) , 27- 40 .
- Weil, C.S. (1952). Table for convenient calculation of medium effective dose (LD₅₀ or ED₅₀) and instruction in its use. Biometrics, 8 , 249 – 263 .
- Zaghloul, M.S. and A.A. El-Daly (1996). Histological and histochemical studies on the effect of some treatments (hydrogenation and ixing) of plam oil on the liver of albino rats. J. Egypt. Ger. Soc. Zool., 19 (c) , 99 – 125 .

التأثيرات الهستوباثولوجية لمبيد القوارض المضادة للتجلط الوارفارين والكلوروفاسينون على ذكور الجرذ الألبينو
حسن إبراهيم الديب ، وحيد محمد جبر ، فاطمة كامل خضر ،
إبراهيم عبد الفتاح العسيلي
معهد بحوث وقاية النباتات - مركز البحوث الزراعية - دقي - جيزة

تم دراسة التأثيرات الهستوباثولوجية لمبيد القوارض المضادة للتجلط الوارفارين والكلوروفاسينون في الكبد والكلية والخصية على ذكور الجرذ الألبينو بعد ١٥ يوم من المعاملة عن طريق الفم بجرعات تحت مميتة مقدارها ١٠/١ ، ٤/١ من قيمة الجرعة المميتة للنصف LD₅₀ . وأظهرت الدراسة من خلال الفحص الميكروسكوبي أن تأثير المعاملة بهذين المركبين على هذه الأعضاء كان واضحا ويعتمد على مستوى الجرعة المعطاة. بالنسبة للكبد، أوضحت النتائج أن كلا المركبين تسبب في حدوث اتساع وتهتك في جدر الأوعية الدموية و الوريد المركزي و البابي مع حدوث احتقان وأرتشاحات ليمفاوية ونخر في الخلايا . وفي حالة الكلية أحدثت المعاملة بكلا المبيدين تضخم كثيف واحتقان مع إرتشاح ليمفاوي و ظهور بؤر نزيفية في الأنسجة الضامة للأنايب الداخلية مع حدوث نخر واضح في الخلايا . أما بالنسبة لتأثير المعاملة بكلا المركبين على الخصية ، فقد أظهرت المعاملة حدوث تغلط في سمك الجدار القاعدي وكذلك حدوث إرتشاح ليمفاوي في الأنايب المنوية .