

## HISTOPATHOLOGICAL CHANGES IN KIDNEY OF NORWAY RAT TREATED WITH ETHANOLIC OSHAR LEAVES EXTRACT

IBRAHIM, K. I.

Plant Protection Research Institute, ARC, Egypt

(Manuscript received 27 June 2005)

### Abstract

The effect of  $\frac{1}{4}$  LD<sub>50</sub> (23.15 mg/kg b.w.) oral administration of ethanolic Oshar leaves extract for 2, 4, 6 and 8 days on body weight and hematological parameters of Norway rat *Rattus norvegicus* including Red blood cell R. B. Cs, white blood cell W. B. C.s and hemoglobin were investigated. The Oshar extract caused a significant decrease in body weight and a significantly increase in white blood cell W. B .Cs, while red blood cells R. B. Cs and hemoglobin significantly decreased. Histological changes of kidney were shown in the form of Hyperemia in the glomerular tuft of the glomeruli and intertubular blood vessels. While Focal extravasation of red blood cells R. B. Cs and hemosiderin pigments between the degenerated renal tubules at the Corticomedullary junction, In addition Mononuclear leucocytes inflammatory cells infiltration in the perivascular area surrounding the dilated blood vessels in between the degenerated renal tubules at the cortex.

However, it was found that the use of sublethal doses of ethanolic Oshar leaves extract has greatly affected the blood picture and kidney tissue of rats.

### INTRODUCTION

Man has been combating pests across much on the earth for many years. His controls efforts have taken numerous forms of synthetic pesticides, and many have been the attempts to get a better way for controlling pests. However, Egypt government has been exerting great efforts and a lot of money to get several synthetic pesticides which soon became more commonly used for controlling the different pests which have become resistant to it. So, the natural products has been recently attracting the attention of many scientists, to avoid the synthetic compounds. They have deeply interested in their chemical constituents and biological properties.

### MATERIALS AND METHODS

#### Plant Material:

Oshar leaves *Calotropis procera* were collected from plants growing widely in fields. Identification were based mainly on the taxonomic characters detailed by Tockholm (1956).

#### Preparation of the crude extract:

The used of 150 gm. of Oshar leaves, was air dried (which were) ground and sieved through 400 $\mu$  sieve. Leaves ground separately macerated consecutively in two

solvents varied in their polarity hexane and ethanol at rate of 5ml solvent/ gm plant material. After 72 hours the extracts were filtered through Buchner funnel to remove debris. Solvents were evaporated under vacuum at 50° c. The crude extract was then weighed and adjusted to 10 ml with the solvent used and kept in a refrigerator until testing (Freedmen *et al*, 1979).

**Tested animals :**

A group of Norway rat, *Rattus norvegicus* were housed under normal conditions. Five rats served as controls while the rest were orally administered  $\frac{1}{4}$  LD<sub>50</sub> Oshar leaves ethanol extract (23.15 mg/k. b.w) as a single dose. The treated rats were sacrificed after 2, 4, 6 and 8 days post-treatment. The collected samples of rat blood from control and treated were processed for carrying out different hematological studies. Red blood cell count (R. B. Cs), white blood cell count (W. B .Cs) and hemoglobin content were determined according to Miller (1960) and Levinson and Mac Fate (1956), respectively. Pieces of examined kidney were fixed in Bouin's for histological study. They were then processed in paraffin wax for microtomy in sections of 5 $\mu$  thick and staining was carried out using haematoxylin and eosin method. (Conn and Darrow 1960). Statistical analysis was done according to Snedecor and Cochran (1967).

### RESULTS AND DISCUSSION

Table (1) revealed that the body weight of treated animals was significantly decreased than that of untreated ones (184.4, 183.9, 181.3 and 175.6 g) at 2, 4, 6, and 8 days post-treatment compared to untreated animals (200.0 g). Also, a significant increase occurred in the weight of kidney following Oshar leaves ethanol extract treatment at 2, 4 and 8 day post-treatment, while at 6 days there was a non-significant increase in kidney weight. It was clear from the present results that the losses in body weight of rats after administration.

Table 1. Effect of Oshar leaves ethanol extract on body and kidney weight of *R. norvegicus* treated with  $\frac{1}{4}$  LD<sub>50</sub>

parameter	untreated mean ±S.E	days post-treatment mean ±S.E			
		2	4	6	8
Body Weight (g)	200.0 ± 1.45	184.4** ± 1.61	183.9** ± 1.60	181.3** ± 1.60	175.6*** ± 1.41
Kidney (g)	1.96 ± 0.04	2.80** ± 0.09	2.38** ± 0.17	2.20 ± 0.28	2.15* ± 0.09

\* Significant at  $p > 0.05$ \*\* Significant at  $p > 0.01$ 

of Oshar leaves extract may be due to the loss of appetite. The same observation was noticed by Sebaili (1996) and Gabr *et al* (2004) who found that Oshar ethanol extract reduced body weight of treated animals. Concerning the haematological pattern, data present in Table (2) revealed significant decreases in red blood cell count of, *Rattus norvegicus* giving  $\frac{1}{4}$  LD<sub>50</sub> of Oshar leaves ethanol extract where 4.84, 4.14, 4.76 and 5.1 million/mm<sup>3</sup> blood at 2, 4, 6 and 8 days post-treatment, respectively. At the same time, white blood cell W. B. Cs count showed a significant increase all over the tested periods where 10.0, 10.6, 10.8 and 9.7 thousand /mm<sup>3</sup> blood. The same trend was observed in Hb content indicating significant changes where 16.1, 16.2, 16.35 and 16.42 when measured at 2, 4, 6 and 8 days post-treatment, respectively. The observed decrease in red blood counts of rat after oral administration of  $\frac{1}{4}$  LD<sub>50</sub> Oshar leaves extract maybe attributed either to the depressive action of Oshar extract on the haemopoietic system or to direct destructive effect on red blood cells. Also, the decrease in red blood cells (R. B. Cs) count recorded in the present study is similar to the results reported by Kumar and Saxema(1991), Hiriashi *et al* (1988) Sebaili (1996). Fatma, Khidr (2003) and EL-Mahrouky *et al* (2003).

Table 2. Effect of Oshar leaves ethanol extract on blood picture in rat treated with  $\frac{1}{4}$  LD<sub>50</sub>.

Parameter	Untreated mean $\pm$ S.E.	Days post-treatment											
		2		4		6		8					
		Mean $\pm$ S.E.	% diff.	Mean $\pm$ S.E.	% diff.	Mean $\pm$ S.E.	% diff.	Mean $\pm$ S.E.	% diff.				
R. B. Cs million/ $\mu\text{m}^3$ blood	5.48 $\pm$ 0.04	4.84 $\pm$ 0.05	-11.7**	4.14 $\pm$ 0.05	-24.7**	4.76 $\pm$ 0.05	-13.5**	5.1 $\pm$ 0.07	-7.3*				
W. B. Cs thousand/ $\mu\text{m}^3$ blood	9.30 $\pm$ 0.06	10.0 $\pm$ 0.06	7.5*	10.60 $\pm$ 0.11	14.0**	10.80 $\pm$ 0.07	16.0**	9.70 $\pm$ 0.07	4.3*				
Hb (gm/100ml blood)	17.20 $\pm$ 0.15	16.1 $\pm$ 0.20	-6.4**	16.2 $\pm$ 0.07	-5.8**	16.35 $\pm$ 0.03	-4.9*	16.42 $\pm$ 0.04	-4.5*				

\* Significant at  $p > 0.05$ \*\* Significant at  $p > 0.01$



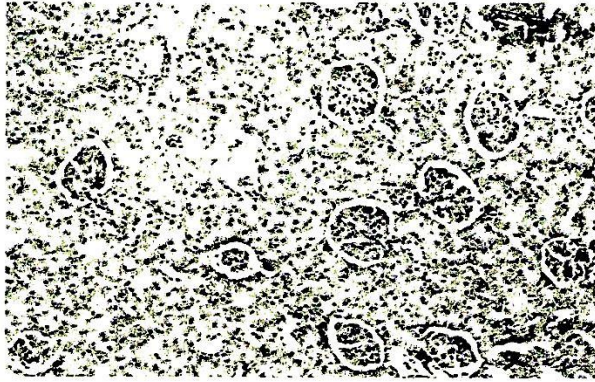


Fig 1. Kidney of rat in control gp showing the normal histological structure of the glomeruli and renal tubules.

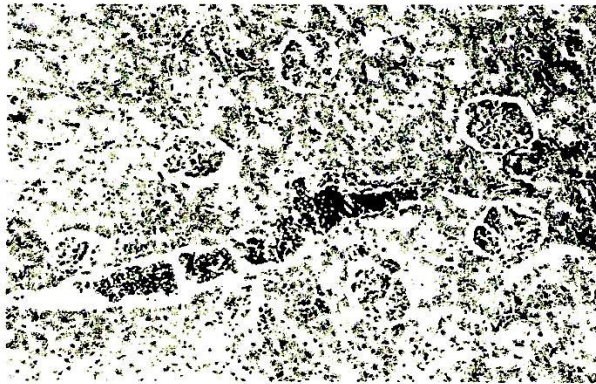


Fig 2. Kidney of rat two days after treatment showing hyperemia glomerular tufts of the glomeruli and intertubular blood vessels in the cortical portion.

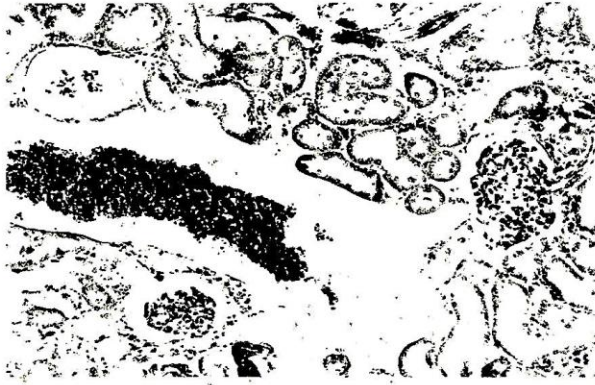


Fig 3. Kidney of rat four days post-treatment showing hyperemia in the glomerular tufts of the glomeruli and in the intertubular blood vessels of the cortex .

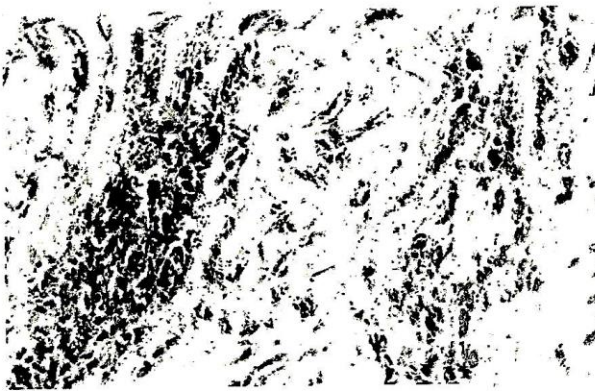


Fig 4. Kidney of rat four days post-treatment showing focal extravasation with hemosiderosis in the corticomedullary portion in between the degenerated renal tubules.



Fig 5. Kidney of rat six days post-treatment showing perivascular mononuclear leucocytes inflammatory cells infiltration with degeneration in the renal tubules and dilation in the blood vessels in the cortex.

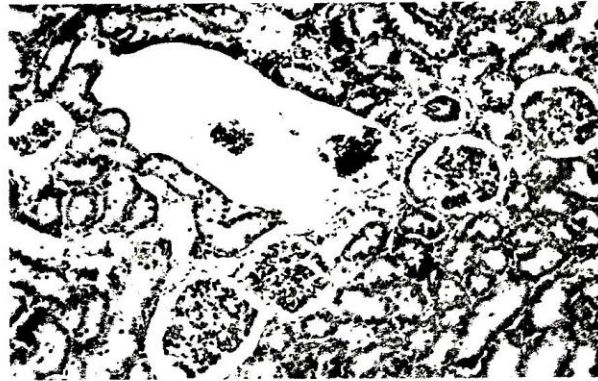


Fig 6. Kidney of rat eight days post-treatment showing hyperemia in the glomerular tufts of the glomeruli as well as the intertubular blood vessels in the cortical portion .





Fig 7. Kidney of rat eight days post-treatment showing extravasation red blood cells surrounding the Bowmans capsule with hyperemic glomeruli.

Histopathological changes in kidney tissue after two days post-treatment with  $1/4$  LD<sub>50</sub> ethanolic Oshar extract are shown in Fig (2) and revealed that the Hyperemia was observed in the glomerular tuft of the glomeruli and intertubular blood vessels. While after four days post-treatment Fig (3) revealed that the hyperemia in the glomerular tufts of the glomeruli as well as in the intertubular blood vessels at the cortical portion. Also, Focal extravasation of red blood cells and hemosiderin pigments were detected in between the degenerated renal tubules at the corticomedullary junction (Fig 4).

In addition (Fig 5) illustrated Mononuclear leucocytes inflammatory cells infiltration was observed in the perivascular area surrounding the dilated blood vessels as well as in between the degenerated renal tubules at the cortex when observed six days post-treatment while after treatment with 8 days marked hyperemia in the glomerular tufts of the glomeruli as well as in the intertubular blood vessels at the cortical portion (Fig. 6) was noticed. Focal extravasation of red blood cells was detected surrounding the Bowmans capsule of the glomeruli (7). These findings are in agreement with. That of Kumor and Saxema (1991), Hiriashi *et al* (1988), Sebail (1996), EL-Deeb, *et al* (2003) and EL-Mahrouky *et al* (2003).



**REFERENCES**

1. Conn, H. J. and M. A. Darrow. 1960. Stain staining procedures used by the Biological stain commission, 2, Geneva, N. Y. Biotech. Publications.
2. El-Deeb, H. I., W. M. Gabr, Fatma, K. Khidr and E. A. El-Essely. 2003. The histopathological effects of Warfarin and Chlorophacinone anticoagulant rodenticides on male albino rat, *J. Agric. Sci. Mansoura Univ.*, 28 (7): 5685-5688.
3. El-Mahrouky, Fatma S., Fatma, K. Khidr, S. M. Abd El-All and H. A. Zedan. 2003. Histopathological effects herbicide (Machete) on the kidney of albino rat. *J. Agric. Sci. Mansoura Univ.*, 28 (6): 5043-5051.
4. Fatma, K. Khidr 2002. Histopathological changes in heart and lung of albino rat treated with Machete herbicide. *J. agric. Sci. Mansoura Univ.*, 27 (10): 7043-7052.
5. Freedman, B., J. No wak and W. F. K. Kwolek. 1979. Abioassax for plant derived pest control agent using the European comborer. *J. Econ. Entomol*, 72: 45-54.
6. Gabr, W. E., Fatma. K. Khidr and A. M. Hegab. 2004. Evaluation of Oshar crude plant extract as a rodenticide under laboratory and field conditions. *Zagazig. J. Agric. Res.*, 31 (4 A): 1607.
7. Hirashi, M., Z. Yamazaki, K. Ichikawa, F. Kanai, Y. Idezuki, K. Onishi, T. Takahama and N. Inoue. 1988. Plasma collection using nafamost at mesilae and dipyrindanole as an anticoagulant. *Int. J. Artif. Organs.*, 11 (3): 212-216.
8. Kumar, D. and Y. Saxena. 1991. Effects of an anticoagulant rodenticide (Brodifacoum) on the haematological aspects of albino mice. *Indian. J. Comp. Anim. Physiol.* 9 (2): 81-86 (e. f. B. A. 94 (2), 23457, 1992).
9. Levinson, S. A. and R. P. Mac Fate. 1956. *Clinical laboratory diagnosis*. 5<sup>th</sup> ed., 124pp., Philadelphia, Ian and Febiger.
10. Miller, S. E. 1960. *A text book of clinical pathology*. 6<sup>th</sup> ed., the williams and wilkins, Battimore, U. S. A.
11. Sebaei, M. AB. M. 1996. Evaluation of the efficacy of some new compounds as pesticides Ph. D. Ithesis. Dept. Zool. Fac. Agric. Alex. Univ. Egypt. 266.
12. Snedecor, G. W. and W. G. Cochran. 1967. *Statistical Methods*. 6<sup>th</sup> ed. oxford and publishing company, Delhi, 593 pp.
13. Tockholm, V. 1956. *Studies flora of Egypt*. Published by Anglo. Egyptian shop, Cairo.

التغيرات الهيستوباثولوجية في أنسجة كلية الفأر النرويجي المعامل  
بمستخلص أوراق العشار الإيثانولي

إبراهيم قطب إبراهيم

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - الجيزة - مصر

أجري هذا البحث بهدف دراسة تأثير ٤/١ الجرعة النصف مميتة (٢٣,١٥ ملجم / كم من وزن الجسم) من مستخلص أوراق العشار الإيثانولي عن طريق الفم لمدة ٢، ٤، ٦، ٨ أيام علي كل من وزن الجسم وبعض قياسات الدم للفأر النرويجي والتي تشمل كرات الدم الحمراء وكرات الدم البيضاء وقد وجد أن هناك نقص معنوي في أعداد كرات الدم الحمراء والهيموجلوبين. أما بالنسبة للتغيرات الهيستوباثولوجية في الكلية كما أوضحت النتائج أيضا وجود احتقان في الأوعية الدموية في الجزء الخارجي من الكلية أما بالنسبة للخلايا الظلالية المبطنة للأنيبيبات الكلوية فقد لوحظ وجود تغيرات إنتكاسية مع وجود إرتشاح والتهابات في الخلايا البؤرية وحيدة النسواه وانتفاخات كلوية .