ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Coragen (Chlorantraniliprole) Insecticide Effects On Male Albino Rats.

Shallan Magdy A. [1], Abdel-Mobdy Yasmin E. [2] and Hamdi Eman [1], and Abdel Rahim Emam A. [1].

[1]Biochemistry Department, Faculty of Agriculture Cairo University P. Box 12613, Gamma St, Giza, Egypt.
[2]Department of Entomology and Pesticide, Faculty of Agriculture, Cairo University, P.O. Box: 12613, Giza, Egypt.

ABSTRACT

The study aimed to evaluate toxic effects of insecticide Coragen (Chlorantraniliprole) doses of 1/20, 1/40 and 1/60LD_{50} orally on albino rat males during experimental period (every 2 consecutive days for 90 days). Rats which administered coragen showed significant decrease in body weight gain and daily body weight gain. Organs weight (heart, kidney and brain) were decreased but spleen and liver showed a significant increase compared with control. Also, there were significant increase in AST and ALT activities, significant changes in the haematological indices RBC count, haemoglobin percentage, haematocrit and WBC count. Also, a slight increase in urea was recorded but creatinine almost remains stable in all intoxicated rats. Coragen 1/20LD_{50} rats showed mild histopathological changes in liver and kidney compared to control. The results of the present study advice to avoid exposure to any pesticide however how much it is safe to avoid hazard risk.

Keywords: Chlorantraniliprole, Coragen, AST, ALT, urea, creatinine, haemoglobin RBC, WBC.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Pesticides are widely used by farmers for agricultural purposes. It has conferred immense benefits to mankind by improving health and nutrition. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans [1]. Pesticides are considered as a source of pollution to aquatic environment. It includes large variety of chemical nature and biological activity grouped together only on the basis that are used to destroy or eliminate the pests [2].

Coragen 20 SC is a new pesticide product containing the active substance chlorantraniliprole, developed worldwide by DuPont with high biological activity. It is applied for use in many crops against all Lepidoptera and some Coleoptera, Diptera and Isoptera species, such as codling moth, apple1 fruit moth and free leaf living larvae. It has very low mammalian toxicity and selectivity to non-target arthropods. The differential in selectivity which chlorantraniliprole has towards insect ryanodine receptors, explains the outstanding profile of low mammalian toxicity [3] .Chlorantraniliprole is a new insecticide belongs to anthranilic diamide class. It is a potent and selective activator of insect ryanodine receptors which are critical for muscle contraction (Lahm et al., 2007)[3], leading to feeding cessation, lethargy, muscle paralysis and ultimately death of the insect [4].

The effect of subacute toxicity of 21-day oral exposure to Coragen and its subsequent analysis of haematological parameters. The results revealed that after 14 and 21 days of exposure, there were significant haematological changes in comparison to the control group. From the entire study it can be concluded that subacute oral administration of Coragen at this particular dose of 1000 mg/kg body weight causes deleterious toxic effect on various haematological parameters in rats [5]. Also, coragen caused severe haemato-toxicity as well as hepato-renal toxicity [6] . The aim of this study was to investigate the toxic effect of Coragen on some haematological parameters, kidneys and liver functions also to examine the histological findings in some organ injuries induced by Coragen administrated into male albino rat.

MATERIALS AND METHODS

Chemicals

Coragen 20% SC (Chlorantraniliprole). It was provided by DU PONT DU NEMOURS Company.

Experimental animals:

This study has been done using a total of sixty male albino Spargue-Dawely rats with a mean weight of 100 ± 20 g. The animals were provided by the animal house of the National Research Centre, Dokki-Giza, Egypt. They were raised in the animal house of Biology Laboratory, Collage of Agriculture, Giza, Egypt. The animals were divided into12 equal groups of 5 rats each and housed individually in stainless steel cages with wire mesh bottoms and maintained at 25 ± 2 °C, relative humidity of 50-60% and 12/12 h light/dark cycle throughout the experiment for one week before the initiation of the experiment and animals were allowed free access of water and basal diet which prepared according to the National Research Council [7].

Animal diet

Basal diet was prepared according to A.I.N. 93M, [8] .Composition of basal diet g/ kg: casein 140; corn starch 465.7; sucrose 100; soybean oil 40; cellulose 50; mineral mixture 35; vitamin mixture 10; L. cystine 1.8; choline chloride 2.5 and Tert. Butylhydroxy quinone 0.008.

Design of the biological experiments

After the adaptation period, 20 of the male rats were divided into 4 groups (5 rats each) as follows (LD_{50} ≥5000 mg/kg body weight by Lahm [9].

Group 1(Control) normal rats were fed on the normal basal diet which used as normal healthy control. Group 2, intoxicated rats were fed with normal diet and ingested orally with 1/20 LD_{50} of coragen

(s20) 250 mg /kg body weight every 2 consecutive days. Group3, intoxicated rats were fed with normal diet and ingested orally with 1/40 LD_{50} of coragen (s20)125mg /kg body weight every 2 consecutive days. Group4, intoxicated rats were fed with normal diet and ingested orally with 1/60 LD_{50} of coragen (s20) 84mg /kg body weight every 2 consecutive days.

Preparation of blood samples

At the end of the experiment period 12 weeks, interval, rats were fasted overnight and then the animals were killed by decapitation. Five blood samples were collected from rats of each group in clean dry sterile and labeled centrifuge tubes. Separating serum was done by centrifugation at 3000 xg for 5 min.

Biochemical determinations:

Hemoglobin was analyzed by the colourimetric method according to Drabkin and Austin [10]. Measurements of red and white blood cell count were done according to the routine methods adopted by Schalm [11]. Measurement of haematocrit (HCT %) estimated by micro haematocrit method according to Schalm *et al*. [12].

Activities of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) in serum were estimated by the colorimetric procedure according to Reitman and Frankel [13] using kits developed by Reactivos GPL Kits, Barcelona, España. Serum creatinine was analyzed by the colorimetric procedure according to Houot [14] using kits developed by Diamond Diagnostics, Egypt. Serum urea was analyzed by enzymatic colorimetric procedure according to Fawcett and Scott [15] using kits developed by Diamond Diagnostics, Egypt.

Pathological examination

Autopsy samples were taken after careful postmortem examination from the liver and kidney rats in different groups and fixed in 10% buffered neutral formaline for 24 hours. Processed for paraffin wax embedding with the automatic tissue processor (SAKURA FINE TECH, Netherlands) by dehydrating through 70%, 90%, 95% and two changes of absolute ethanol for 90 min each. Clearing was achieved through changes of xylene twice for two hours each, infiltrating through two changes of paraffin wax at 70°C and embedded in paraffin wax. Sections were cut at 4 µm with the rotary microtome (SAKURA FINE TECH, Netherlands) and mounted on glass slides and dried at 65°C for 45 min, then stained with hematoxylin and eosin stain, then examined by the light microscope Carleton et al. [16].

Statistical analysis:

Statistical analysis standard error ‘‘SE’’ was carried out according to Fisher [17]. LSD (Least signific6ant difference) test was used to compare the significant differences between means of treatment Waller and Duncan, [18]. The Costat program was used for all analysis.

RESULTS AND DISCUSSION

The present investigation was designed to study biological and biochemical effects of coragen harmful .The obtained results could be showed and discussed under the following titles.

Biological and biochemical effects of coragen

Body weight

The effect of coragen with doses of 1/20LD_{50}, 1/40LD_{50} and 1/60LD_{50} were inducted into albino male rats orally during experimental period which was 90 days. Results in Tables (1) summarize the mean values of initial and final body weight, body weight gain and daily body weight gain.

A significant decrease was observed in body weight gain and daily body weight gain in (Table. 1) for experiment when rats coragen with respective controls,($P<0.05$).

ISSN: 0975-8585

Table (1): Average (mean ±S.E.) performance value (g) of rats received coragen orally for 90 days

Group/ dose	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Daily body weight gain (g)
Group1 control	99.01±5.32	126.51^{a}±3.17	27.50^{a}±5.77	0.31^{a}±0.06
Group2 (1/20 LD_{50})	98.60±5.73	107.57^{d}±7.83	8.63^{d}±2.15	0.10^{d}±0.02
Group3 (1/40 LD_{50})	99.06±3.97	112.10^{c}±3.88	13.04^{c}±1.30	0.14^{c}±0.01
Group4 (1/60 LD_{50})	97.41±1.98	115.07^{b}±6.08	17.66^{b}±4.16	0.20^{b}±0.05

Each value represents the mean ± S.E (Standard Error) and mean of five replicates to each sample. Values in the same column with the same letter are not significant at p≤0.05

A study reported that reductions in mean body weight (92% of values for controls) and body-weight gain (43% of values for controls) were observed in males at the highest dose over the 28 days. It was noted that the body-weight gain of the mice in the control group varied greatly during this period, with an initial drop in weight during the first week, followed by a rapid recovery during the following 3 weeks. Weight gain in the groups receiving chlorantraniliprole was constant throughout the treatment period. No dose-dependent effect on body-weight gain was observed in the treatment groups [19].

In coincidence with the reports of Luckett [20] that dogs exposed to chlorantraniliprole at any dietary concentration. Cause a 20% reduction in body weight, the 20% reduction in body weight was only experienced in study weeks 11 and 12. the results of the present study clearly demonstrated that, oral administration of coragen to male rats induced significant reduction of body weight.

Organs weight

Tables (2) showed the effects of coragen on rats relative organs weight. The results of organs weight showed significant decrease in heart , kidney and brain and relative weight in the experimental groups compared with respective controls groups (P<0.05).While liver and spleen showed a significant increase relative weight in all experimental groups compared with respective controls (P<0.05) in Table (2).

Table 2. Effect of coragen on rats relative organs weights for 90days.

Group/ dose	Liver	%	Brain	%	Heart	%	Kidney	%	Spleen	%
Group1 (control)	$3.43±0.30^{c}$	100	1.70^{a}±0.06	100	0.90^{a}±0.01	100	0.78^{d}±0.05	100	0.32^{c}±0.01	100
Group2 1/20LD_{50}	$5.19±0.1^{a}$	147	1.29^{c}±0.05	76	0.46^{b}±0.06	51	1.43^{a}.0.07	183	0.67^{a}±0.03	209
Group3 1/40LD_{50}	$4.41±0.66^{b}$	125	1.42^{b}±0.07	84	0.50^{b}±0.02	56	1.13^{b}±0.06	145	0.56^{b}±0.04	175
Group4 1/60 LD_{50}	$4.30±0.22^{b}$	121	1.48^{b}±0.1	87	0.54^{b}±0.08	60	0.98^{c}±0.01	126	0.55^{b}±0.05	171

Each value represents the mean ± S.E (Standard Error) and mean of five replicates to each sample. Values in the same column with the same letter are not significant at p≤0.05

These results are in covenant with the results Luckett [20] who observed in dogs exposed to chlorantraniliprole at any dietary concentration. a 20% reduction in body weight and a decrease of approximately 25% in heart weight were reported for females in the group at 10 000 ppm.. On the basis of the lack of dose–response for both body weight and heart-weight effects, these effects were not considered to be treatment-related. Increases in relative liver weights (up to 26%), were observed in male dogs in all groups receiving chlorantraniliprole. At 40 000 ppm, the increase (26%) was statistically significant.

Also, another study observed a slight increase in mean liver weight in 3000 and 7000 ppm females and a mild increase in cytochrome P450 content observed in males and females at 3000 or 7000 ppm .Decreased hepatic β-oxidation activity in males at the highest dose (79% of values for the controls) and females (54% of values for the controls) [19] . These results conformed with Donner [21] who observed that the mean relative kidney weights were statistically significantly higher than the control in all female exposure

groups. In coincidence with the report of Gannon [22] was observed a slight increase in liver weight in the group at 7000 ppm in an 18-month study in mice (9–17%).

Effect of coragen on some haematological parameters

The effect of coragen with doses of $1/20LD_{50}$, $1/40LD_{50}$ and $1/60LD_{50}$ were inducted into albino male rats orally during experimental period which was 90 days were studied. Results in Tables (3) summarize the values of haematological parameters like haemoglobin content, red blood cells count, the white blood cells count and haematocrit . The results showed significant decrease in hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs) and haematocrit (HCT) experimental groups compared with respective control group ($P<0.05$).

Table3. Changes in the haematological parameters of rats exposed to Coragen (for 90 days).

Groups/dose	Hb (g/dl)	RBCs count (10^6/uL)	HCT (%)	WBCs count ($10^3/mm^3$)
Group1	13.35 ± 0.76^a	4.45 ± 0.25^a	$40.40^a \pm2.28$	$6233.33^a\pm260.34$
Group2	11.33 ± 0.34^b	3.78 ± 0.11^b	$33.98^b\pm1.03$	$4040.67^c\pm144.64$
Group3	11.39 ± 0.21^b	3.80 ± 0.07^b	$34.18^b\pm0.63$	$4833.33^b\pm88.19$
Group4	11.58 ± 0.19^b	3.95 ± 0.06^b	$35.55^b\pm0.57$	$4933.33^b\pm218.58$

Each value represents the mean ± S.E (Standard Error) and mean of five replicates to each sample. Values in the same column with the same letter are not significant at p≤0.05

Effect of coragen induced haematological changes which cause significant changes in the haematological indices (red blood cell (RBC) count, haemoglobin percentage, haematocrit, mean corpuscular volume of RBCs, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and white blood cell (WBC) count) were observed [6].

Also another study [5], observed that Coragen exposure showed significant decrease in the erythrocyte count (RBCs), haemoglobin percentage, haematocrit percentage and leukocyte count (WBCs). The decrease in RBC count and Hb % can be correlated with the increased arterial O2 saturation of blood, which acts indirectly as stimulus for bone marrow erythrocyte production [23]. The significant decrease in the RBC, haemoglobin and haematocrit may be a consequence of severe haemorrhage which results in the dilution of blood caused due to the influx of cells and fluids from body stores [24, 25]. In the severe anaemic condition, there is immense decrease in the number of red blood cells leading to impaired synthesis of haemoglobin due to iron deficiency or impaired production of erythrocyte due to deficiency of folic acid and vitamin B12 [26].

The decrease in WBCs count was due to the possible reason of their getting used up while encountering a variety of inflammation injury and subsequent infections resulting due to the Coragen treatment. This decrease is possibly due to the failure or suppression or destruction of stem cells in the bone marrow, which leads to decrease in the number of leucocytes denoting marked decrease in the cellularity of bone marrow [27, 28].

Kidneys and liver functions

Effect of coragen administration on serum AST and ALT activity in normal and coragen exposed rats is presented in table (4), intoxicated rats showed a significant increase in serum AST and ALT activity all over the periods of the experiment when compared with normal control group.

Table4. Coragen toxicity on liver function of the experimental rats.

Groups/dose	AST activity (U/L)	%	AST activity (U/L)	%
Group1	35.21 ± 0.61^d	100	35.87 ± 0.51^d	100
Group2	71.90 ± 2.03^a	202	67.19 ± 2.16^a	192
Group3	60.53 ± 1.19^b	170	58.59 ± 3.01^b	168
Group4	49.07 ± 0.50^c	138	51.63 ± 4.20^c	144

Each value represents the mean ± S.E (Standard Error) and mean of five replicates to each sample. Values in the same column with the same letter are not significant at p≤0.05

These result are in agreement with Dutta et al. [6] who stated that coragen caused severe hepatoxicity . Salim *et al.* [29] observed that other pesticide caused high significant increase in liver function (ALT and AST activities) compared to control animals also confermed with Saafi *et al*.[30].

Serum AST and ALT activities are the most sensitive biomarkers used in the diagnosis of liver diseases Pari and Kumar [31]. The affected liver function of coragen was similar to that reported by El-Damaty *et al*. [32] . Elevated serum ALT and AST activities have been reported as a sign of liver cell damage [33].

Effect of coragen administration on serum urea and creatinine concentration in rats is presented in table (5). coragen intoxicated rats showed a significant increase in serum urea values all over the periods of the experiment when compared with normal control group.

Table5. Coragen toxicity on kidney function of the experimental rats.

Groups/dose	Urea(mg/dl)	%	Creatinine (mg/dl)	%
Group1	36.55 ±1.01d	100	0.94 ±0.05a	100
Group2	49.26 ±1.09a	138	1.21 ±0.10a	124
Group3	45.55 ±0.51b	128	1.14 ±0.30a	117
Group4	40.21 ±0.33c	113	1.03 ±0.17a	105

Each value represents the mean ± S.E (Standard Error) and mean of five replicates to each sample. Values in the same column with the same letter are not significant at p≤0.05

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore, marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney [34].

Exposure to pesticides has been reported to evoke an imbalance cellular oxidative status and cause tissue injury including lipid peroxidation, DNA damage and enzyme in activation [35]. These results are in agreement with Dutta *et al*. [6] who observed significant increase in urea and creatinine levels noticed were a classical sign that the kidney was adversely affected by coragen administration. Oral administration of pesticides in rats induced a marked renal failure characterized by a significant increase in serum urea levels [29].

Histopathological studies

The Microscopical examination of liver indicated that it was congested with hepatocytes and kidneys have degenerative changes.

Liver

Liver showed normal patent portal area with patent hepatic cards and hepatic sinnsoids (Figure1). Rats treated with coragen dose 1/60 of LD_{50}, liver showed few of congestion of portal blood vessels and hemolysis of RBCs (Figure 2).Rats treated with coragen dose 1/40 of LD_{50} liver showed portal to portal lymphocytic in filtration as well as congestion of portal blood vessels. Kupffer cell activation could be detected (Figure 3). Rats treated with coragen dose 1/20 of LD_{50} liver showed heighly proliferated bile ducts with congested portal blood vessels, some hepatocytes had single cell necrosis (Figure 4). present study revealed variable pathological changes in livers, the severity of the lesions increases with the dose of coragen which were in agreement with those of saafi et al [29], who found that pesticide intoxication exhibited severe histopathological changes such as mononuclear cells infiltration in the parenchymatous tissue and portal area, congestion, enlargement of the hepatic sinusoids and enlargement of the central and the portal veins and hepatocellular damage. Also, Donner and Sykes [21, 36] were observed in the groups at 1500 and 8000 ppm. Minimal centrilobular hepatocellular hypertrophy was observed in females at 8000 ppm. There was no evidence of hepatic cell damage.

Kidney

ISSN: 0975-8585

The histopathological examination of kidney revealed normal histological appearance of control group 1 (Figure 5); While group (2) showed congestion of renal blood vessels and glomerular capillaries with some degenerative changes of renal tubular epithelium (Figure 6); Rats in group (3) which rats treated with coragen dose 1/40 of LD50 showed slight congestion in some renal blood vessels .Slight degenerative changes in some epithelialcells of renal tubules (Figure7); While group (4) which rats treated with coragen dose 1/60 of LD50 showed few congestion of renal blood vessels with haemorrhagic spots (Figure 8).

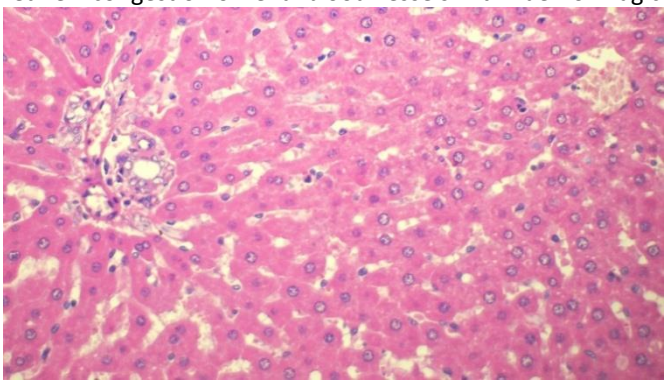


Fig 1 Photomicrographs of liver Control (G1) rat showing normal histological picture. (X 200).H and E stain.

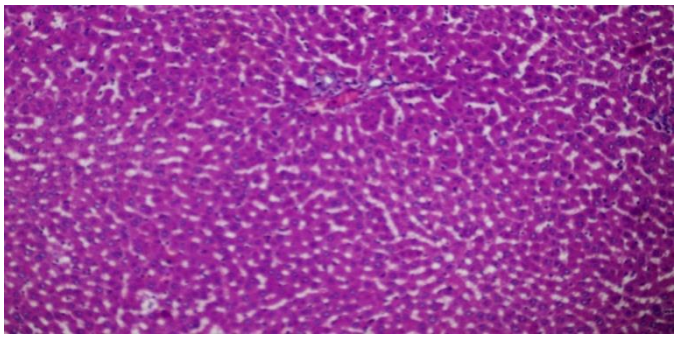


Fig 2 Photomicrographs of liver rats treated with coragen dose 1/40 of LD50 (X 400).H and E stain.

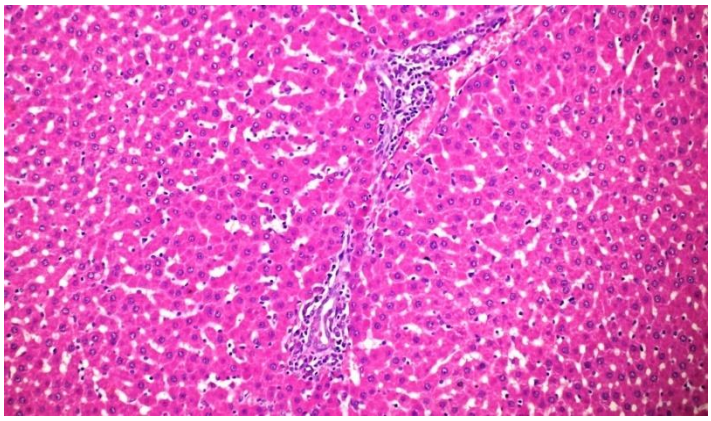


Fig 3 Photomicrographs of liver: rats treated with coragen dose 1/40 of LD50 (X 100).H and E stain.

ISSN: 0975-8585

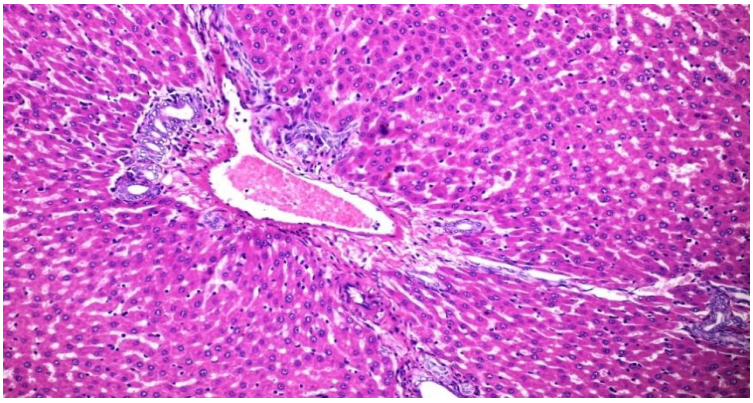


Fig 4 Photomicrographs of liver: rats treated with coragen dose 1/20 of LD50 (X 100).H and E stain.

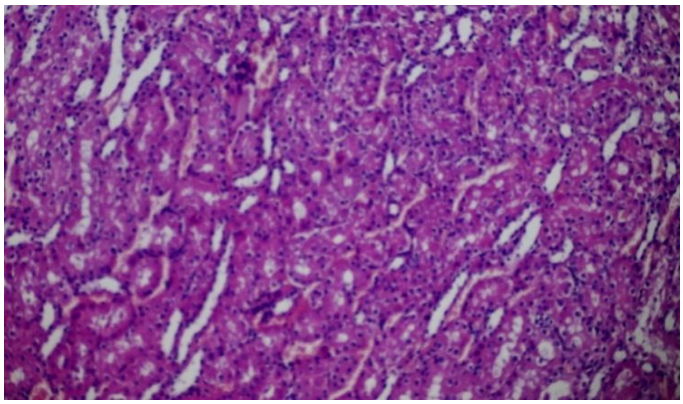


Fig. 5. Photomicrographs of kidney: Control group (1) rat showing normal histological structure of kidney. (X 400).H and E stain.

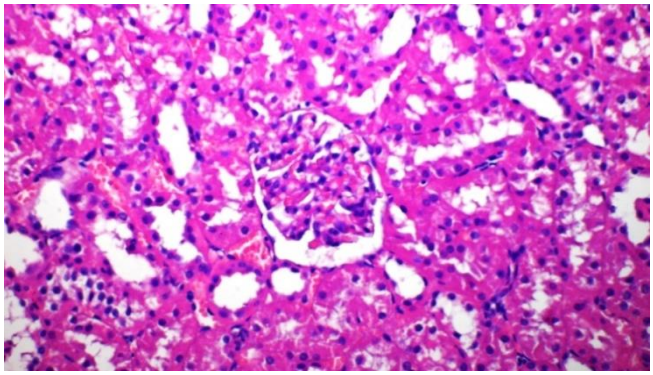
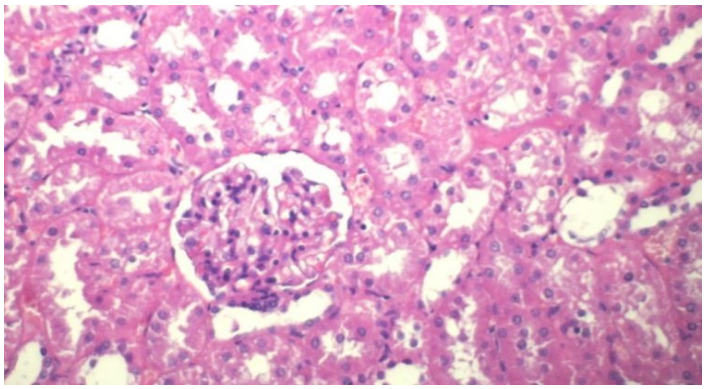


Fig. 6 .Photomicrographs of kidney: rats treated with coragen dose 1/40 of LD50 group (2).(X 400).H and E stain.



ISSN: 0975-8585

Fig.7. Photomicrographs of kidney rats treated with coragen dose 1/40 of LD50 group (2).(X 400).H and E stain.

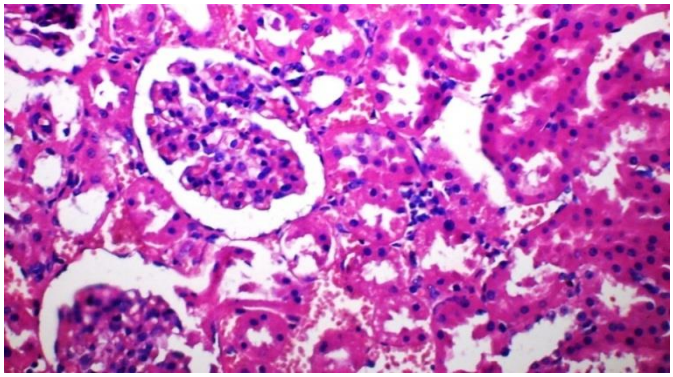


Fig. 8. Photomicrographs of kidney rats treated with coragen dose 1/40 of LD50 (X 400).H and E stain.

CONCLUSION

In conclusion, this study showed the pathological parameters of chronic liver damage, kidneys and heamatoligical changes by Coragen (Chlorantraniliprole), which caused an increase in AST and ALT activities, and changes in the haematological indices (RBC) count, haemoglobin percentage, haematocrit and white blood cell (WBC) count. Also, a slight increase urea was observed. The results of the present work advice the need to avoid exposure of humans to coragen and we recommend that all pesticide no matter how safe it is must assess its toxicity.

ACKNOWLEDGMENTS

The authors would like to thank the management of the Faculty of Agriculture and Cairo University for the ongoing cooperation to support research and for providing the funds and facilities necessary to achieve the desired goals of the research.

REFERENCES

[1] Timothy CM, Ballantyne . Practical Clinical Chemistry. Boston: Little Brown and Co.2004,P 154.
[2] Kamel F, Tanner, C.M., Umbach, D.M., Hoppin, J.A., Alavanja, M.C.R. and Blair, A,. Am J. Epidemiol 2007;165: 364 – 374.
[3] Lahm GP, Stevenson TM, Selby TP, Freudenberger JH, Cordova D, Flexner L. Bioorg Med Chem Lett 2007 ;17:6274–6279.
[4] Cordova D, Benner EA, Sacher MD, Rauh JJ, Sopa JS and Lahm GP. Pestic. Biochem. Physiol. 2006; 84:196–214.
[5] Kumar A, Dutta K, Najam A, Nath A, Singh JK, Ali M, Kumar R . Eur. J. Toxicol. Sci.2013; 4: 1-7.
[6] Dutta K, Ali M, Najam A, Kumar R and Kumar A. J. Toxicol. Sci. 2014; 6(10):194-202.
[7] National Research Council, (1978). National Research council Committee on Animal Nutrition. Nutrient requirement of laboratory animal no. 103rd revised edition. National Academy of Science. National Research Council. Washington, D.C.
[8] Reeves PG ,Nielsen F H and Fahey, Jr.G.C. J. Nutr 1993; 123:1939 –1951.
[9] Lahm GP, Stevenson TM, Selby TP, Freudenberger JH, Cordova D and Flexner L. Med. Chem. Lett. 2007;17:6274–6279.
[10] Drabkin D L and Austin J H. J.Biol. Chem 1935; 112:105-115.
[11] Schalm O W. Veterinary Haematology . Fourth Ed., Lea & Febiger , Safety 1986; 5 : 171 – 176 .
[12] Schalm O W.; Jain, N.C. and Caroll, E.J. Veterinary haematology. 3(ed.) Lea and Fibiger. Philadelphia, USA.(1975); 45-46.
[13] Reitman, S. and Frankel, S. (1957). Am. J. Clin. Path., 28: 56.
[14] Houot O. Interpretation of clinical laboratory tests, Ed. siest, G., Henn, J., Sciele, F. and Young, D.S., Biochem .Publication. ,1985, P.P. 220.
[15] Fawcett, J.K. and Scott, J.E. J.Clin .Pathol 1960; 13: 156-159.

[16] Carleton HM, Druy, R.A.; Willington, E.A. and Coneron's, H.. Histological Technique 4 th Edition. Oxford univ.Press,N. Y. 4, Toranto. 1967.
[17] Fisher R A (1970). Statistical method for research workers Edinburgh ed. 14, Oliver and Boyed, p. 140.
[18] Waller R A and Duncan D B. J. Am. Stat. Assoc.1969; 64: 1485 – 1503.
[19] Finlay, C. (2003) DPX-E2Y45 technical: repeated dose oral toxicity 28-day feeding study in mice. Unpublished report No. 12404 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
[20] Luckett, E.M. (2004) DPX-E2Y45 technical: 90-day oral toxicity study in dogs. Unpublished report No. 12749 from MPI Research, Mattawan, Michigan, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
[21] Donner, E.M. (2006) DPX-E2Y45 technical: subchronic toxicity 28-day feeding study in rats. Unpublished report No. 9523, Revision No. 1 from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA.
[22] Gannon, S.A. (2006) DPX-E2Y45 technical: subchronic toxicity 90-day feeding study in mice. Unpublished report No. 12750, Supplement No. 1, from DuPont Haskell Laboratory, Newark, Delaware, USA. Su bmitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA
[23] Zabulyte D, Vleckiene S, Kalibatas J, Paltanauiciene A, Jascaniniene N, Stosik M Vet. Inst. Pulaury 2007; 51:79-82.
[24] Celik I, Suzek H. Ecotoxicol. Environ. Saf. 2009; 72(3):905-908.
[25] Kalender S, Uzun FG, Durak D, demir F, Kalender Y . Toxicol. 2010;48:633-638.
[26] Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Illustrated Biochemistry, International 26th Edition. The Mcgraw – Hill Companies, Inc. 2007,pp. 46-47, 12.
[27] Chandrasoma P, Taylor CR 1991;1:375-421.
[28] Uzun FG, Kalender Y.Food Chem. Toxicol. 2013;55:549-556.
[29] Salim A B ,Abou-Arab A A K, Mohamed S R and Eldesouky, T.A. 2014; Int. J. Biomelcul. Agric . Food Biotechnol. Eng 2014; 8(8):921-926.
[30] Saafi E B, Louedi M, Elfeki A, Zakhama A, Najjar M F, Hammami M and Achour L .Exp. Toxicol.Pathol 2011;63 (5):433-441.
[31] Pari L and Kumar N A. 2002; J. Med. Food, 5(3): 171-177.
[32] El-Damaty M A ,Abdel-Razik Farrag H, Rowayashed G and Hany Fahmy, M. Q. J.Appl. Sci. Res., 2012;8(11) 5459-5469.
[33] El-Shenawy N S and Abdel-Rahman M S. Toxicol. Letters 1993; 69:77-85
[34] Garba SH, Adelaiye AB, Msehelia LY. J. App. Sci. Res 2007;3:1788-1793.
[35] Dal- pizzol F, Klamt F,Bernard, R J S and Moreira J C F. Free .Radic .Res 2001;35:749-755.
[36] Sykes, G.P. (2006) DPX-E2Y45 technical: subchronic toxicity 28-day feeding study in rats. Unpublished report No. 9523, Supplement No. 1, from DuPont Haskell Laboratory, Newark, Delaware, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, Delaware, USA