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RELATIVE TOXICITY OF RODENTICIDE COUMATETRALYL AGAINST SOME DOMINANT WILD RAT SPECIES FROM FOUR CITIES IN ASSIUT GOVERNORATE

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Rodents are considered as one of the most important pests in Egyptian agriculture. In most countries warfarin and related compounds of anticoagulants are undoubtedly the most commonly used, but resistance to warfarin has increasingly become a big problem. Coumatetralyl (COM) is a multi-dose anticoagulant of the 4-hydroxycoumarin vitamin K antagonist type used as a rodenticide. The current study was designed to determine the dominant rodent species in four sites at Assiut Governorate; Manfalut (1), Deirout (2), Abnoub (3) and Assiut (4). Then the LD_{50} of COM doses for each dominant species were estimated. In addition, the harmful effects of ¼ COM LD_{50} doses on the studied wild rats were determined. Oxidative stress parameters; carbonyl protein (CP), antioxidants such as glutathione (GSH), activities of superoxide dismutase (SOD), and catalase (CAT) were measured in liver, kidney and erythrocytic lysate. Also, prothrombin time (PT), international normal ratio (INR), fibrin degradation product (FDP), clotting time and bleeding time were determined. In addation to the histopathological examination of liver and kidney. The result showed that the dominant species found in the studied four sites were *Rattus rattus*, *Arvicanthis niloticus* and *Gerbillus gerbillus* the LD_{50} of COM estimated in these species were 39.34, 35.29 and 42.84 mg /Kg bw, respectively. Generally, there was a significant rise in CP level and a significant decrease in the antioxidant parameters in the most samples from COM-treated species. Furthermore, the present study showed a significant increase in INR, PTT, clotting time and bleeding

time. However, the FDP levels were < 5 in both control and COM-treated groups. In conclusion, the present results suggested that the ¼ LD50 of COM had very toxic effect as well as powerful susceptibility to the all present studied rodents. So, this used dose could have a potent ability to reduce these pests by lower cost and probably less pollution.

Key Words: Coumatetralyl, Rodenticides, Wild rats, Assiut, Carbonyl Protein and Antioxidants.

INTRODUCTION

Rodents occur worldwide and have adapted to most types of ecosystems. They provide many important ecosystem functions and while most rodent species do not cause serious damage problems, a small number of species do. Rodent-caused damage includes crop and stored food consumption and contamination, forestry and nursery damage, rangeland damage, ornamental plant damage, property damage, cable and irrigation pipe damage, disease transmission, and when introduced to islands, damage and even extinction of native flora and fauna. Many tools are used to reduce rodent populations and damage **(WHO, 1995)**. In Egypt, **El-Abd and Abd El –Hady (2017)** evaluated the field efficiency of three rodenticide baits which they were zinc phosphide 1.5%, COM 0.0375% and brodifacoum 0.005%. Filed trials were carried out in wheat fields at Etay El-Baroud Research Station found that El-Behera Governorate infested with wild *Rattus rattus*. The authors added that the population reduction because of using COM only bait was 63.33% while the efficiency of brodifacoum bait was 67.66 %. However, the efficiency of zinc phosphide bait 1.5%, (crushed maize 98.5%) was only 44.47%.

In most countries warfarin is undoubtedly the most commonly used anticoagulants, but resistance to warfarin has become an increasingly a big problem **(Meehan, 1984).** Rodenticide resistance to anticoagulants in *Rattus norvegicus* will lead to increased difficulties in combating these pest animals **(Meerburg *et al*., 2014).** The authors added that for the last decade, increased

attention had been paid to the emergence of rodenticide resistance among *Rattus norvegicus* in Europe.

The use of the natural anticoagulant, namely diccoumarin as a rodenticide was firstly described by **O´Connor (1948),** since that time this class of rodenticide has become widely used. Their action is cumulative and most of them need to be ingested over a period of several days to be effective. Anticoagulants possess two main advantages over acute rodenticides. First, they are readily accepted by commensal rodents when they are included in bait at low concentration, so that sub lethal dosing and bait shyness problems do not normally arise. Secondly, primary and secondary poisoning hazards to non-target species are generally reduced and if accidental poisoning of man or animals does occur, an effective antidote, phytomenadione (vitamin K) is available.

Warfarin intake resulted in histologically evident tissue damage, leukocyte infiltration and intestinal inflammation increases in myeloperoxidase activity, malondialdehyde content, SOD and CAT activity **(Mirkov *et al.*, 2016).** In *Rattus rattus* when dosed with Racumin 57 showed reduction in hemoglobin (Hb), red blood cells (RBCs), platelets and prolongation of the bleeding time, however, there was an increase in leukocyte count and decrease in platelets **(Helal *et al.*, 1974).** Several coumarins had also been developed as potential antithrombotic and antiplatelet agents **(Frédérick *et al.*, 2005).**

The house rat *Rattus rattus* (Linnaeus) is a native of the Indian sub-continent and now spread throughout the world. It feeds and damages almost all edible things. It is the most abundant, widely distributed, highly adaptable and cosmopolitan commensal rodent species worldwide. *Arvicanthis niloticus* is a rodent of medium size, with the length of the head and of the body between 159 and 202 mm, the length of the tail between 125 and 173 mm, the length of the foot between 33 and 42 mm, the length of the ears between 19 and 23 mm and a weight up to 201 g. The fur is rough. The lesser Egyptian gerbil (*Gerbillus gerbillus*) is distributed mainly in Morocco,

Northern Nigeria to Jordan and Kenya. It is also known as the small Egyptian gerbil. It is much smaller than other gerbils sometimes kept as pets **(Aulagnier *et al.*, 2008).** Generally, the main crops such as wheat, barley, legumes and alfalfa, all of which are liable to attack by desert-adapted, gerbilid rodents **(Bernard, 1977).**

This study was an attempt to determine the dominant wild rodent species in four sites which are **Manfalut (1)**, **Deirout (2), Abnoub (3) and Assiut (4).** In addition, to determine LD_{50} of COM and the toxic effect ¼ LD_{50} of COM aiming to interpret the harmful effect of this dose on these studied wild rodents.

MATERIALS AND METHODS

1) **Survey in the field:**
   Search about the dominant wild rodent species at the four sites in the present study which are **Manfalut (1)**, **Deirout (2), Abnoub (3) and Assiut (4).**

2) **Determination of Abundance (A %) and Dominance degree (D %):**
   All data obtained from survey were subjected to the statistical analysis using F-test according to **Snedecor and Cochran (1971)**, also dominance (D) and abundance (A) degree were determined according to **Facylate (1971).**

$$D = t/T \times 100$$

Where,

t = Total number of each species during the collecting period.

T = Total number of all species collected during the collecting period

$$A = n/N \times 100$$

Where,

n = Total number of samples in each species appeared.

N = Total number of samples taken all over the season.

3) Determination of LD50:

In the present study, general results from the field survey concluded that the dominant trapped species of rodents from the four sites in Assiut Governorate were distributed as *Rattus rattus* was dominant species in Deirout and Abnoub sites, *Gerbillus gerbillus* was dominant species in Manfalut site, and *Arvicanthis niloticus* was dominant species in Assiut site. The acute oral COM LD_{50} of *Rattus rattus* collected from two sites in Assiut Governorate mentioned before was determined. Serial doses of COM measured as mg/kg body weight were prepared as (4, 8, 12 and 16 g). Four adult rats caged individually were used for each dose orally administered in the bait. Animals were fasted for about 12 hours before treatment. Mortality and time to death were recorded up to 4 days after treatment. The LD_{50} values were calculated after 96h by using special tables given by **Horn (1956), and** according to the "Probit analysis" technique described by **Finney (1971).**

4) Effect of ¼ LD50 COM:

Animals of *Rattus rattus*, *Gerbillus gerbillus* and *Arvicanthis niloticus* were trapped from the four sites in Assiut Governorate and kept in a controlled light room with normal photoperiod (dark light cycle 12:12) at a temperature of 30±2 ºC. Rats were randomly divided into six groups, (n=10) to each group. The control groups: The first group was Gerbillus *g.* (CG1), the second group was *Arvicanthis n.* (CA2) and the third group was *Rattus r.* (CR3) were feeding with normal diet and tape water. The treated groups: the fourth group was Gerbillus *g.* (TG1), the fifth group was *Arvicanthis n.* (TA2) and the sixth group *was Rattus r.* (TR3) were feeding diet of wheat containing 0.0375% of COM. and tap water ad libitum.

Doses of ¼ LD_{50} COM for all rat species calculated from **Table (1)** (the ¼ LD_{50} of COM given to *Gerbillus g., Arvicanthis n. and Rattus r.* were 8.82, 10.71 and 9.83 mg/kg bw respectively).

Table (1): The acute oral LD_{50} toxicity of different COM doses against the three dominant studied rodent species collected from the four sites in Assiut Governorate.

Species	No. of rodents in groups	Daily bait consumed (g/day)	Sum bait consumed (g/4day)	COM dose content content of 4 days in the bait (mg/kg b.w.)	No. of died rodents	LD_{50} (mg/kg b.w.)
Arvicanthis niloticus **(150-190 g)**	**4**	**1**	**4**	**8.82**	**0**	**35.28**
	4	**2**	**8**	**17.64**	**0**	
	4	**3**	**12**	**26.46**	**1**	
	4	**4**	**16**	**35.28**	**2**	
Gerbillus gerbillus **(70-90 g)**	**4**	**1**	**4**	**21.42**	**0**	**42.84**
	4	**2**	**8**	**42.84**	**2**	
	4	**3**	**12**	**64.26**	**3**	
	4	**4**	**16**	**85.68**	**4**	
Rattus. Rattus **(90-130**	**4**	**1**	**4**	**13.64**	**0**	**39.34**
	4	**2**	**8**	**27.27**	**1**	
	4	**3**	**12**	**40.91**	**2**	
	4	**4**	**16**	**54.54**	**3**	

- **Chemicals used:**

  Coumatetralyl (Racumin), 5,5 dithiobis-2-nitrobenzoic acid, catalase and superoxide dismutase enzymes standards, guanidine hydrochloride, 1, 2 dichloro-4-nitrobenzen (DCNB) and epinephrine were purchased from Sigma-Aldrich (St Louis, MO, USA) and Fluka companies. All other chemicals and reagents were of the highest purity commercially available**.**

- **Sample collection**

  At the end of experiment 4 days (all period of the experiment), Blood samples were collected by retro-orbital sinus using ether for anesthesia. Blood samples were collected in disodium citrate solution 3.8 % (1 ml citrate solution /9 ml blood) mixed, and then centrifuged at 3000 r. p.m. for 15 minutes. The supernatant plasma was removed and kept under freezing condition until used for determination the prothrombin time (PT). Another blood samples were collected in EDTA tubes for preparation of erythrocytic hemolysate. Immediately after collection, blood samples were centrifuged at 4 rpm for 10 min at 4°C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic phosphate-buffered saline, followed by recentrifugation and removal of the supernatant fluid and the buffy coats. The crude red cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte hemolysate. Animals then scarified and tissues of liver and kidney from control and treated groups were collected, washed with saline solution and immediately kept at -20°C until used for biochemical analysis.

- **Tissue homogenate and Erthyrocytic lysate preparations:**

For preparing 10% w/v homogenate of liver and kidney 500 mg of each were homogenized using homogenizer (IKA Yellow line DI 18 Disperser, Germany), in 5 ml (0.1 M) phosphate buffer (pH 7.4). The homogenates were centrifuged at 5000 rpm for 30 min at 4 °C and the supernatant cytosols were kept frozen at -20 °**C** for the subsequent biochemical assays.

- **Biochemical determinations:**

I- Estimation of carbonyl protein (CP)

It was determined using the method of **Stadtman and Levine (2000).**

II- Determination of CAT activity

CAT activity was measured in the hemolysate, liver and kidney tissues according to the method of **Beers and Sizer (1952).** Decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm.

III- Determination of SOD activity

The activity of SOD in liver and kidney tissues was determined as a result of its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of **Misra and Fridovich (1972).**

IV- Determination of GSH

The concentration of reduced glutathione was measured based on method of **Ellman (1959).**

V- Estimation of total protein

Total protein concentration in the supernatant of liver, kidney homogenate was determined by the method of **Lowry *et al* (1951)** and expressed as mg/ml.

VI- Determination of PT, INR and FDPs

The measurements of PT and INR in plasma samples were carried out at Alborg lab (the branch of Assiut Governorate).

VII- Determination of blood clotting time

Clotting time of blood samples was determined by the capillary tube method. Tail region of mildly anaesthetized rat was punctured. First two drops of blood were discarded. Capillary tube (8 cm in length with diameter 0.8 to 1.2 cm) was held horizontally in the blood drop to rapidly allow the blood to run into non-heparinized capillary tube. Capillary tube was broken off about 1 cm length of tubing after every 30 seconds and recorded the coagulation time as the interval from the time the blood appeared on the skin of rat until a fibrin thread bridged the broken ends of the capillary tube.

VIII- Determination of blood bleeding time

Tail region was catting and immediately turn on the stop watch to take time until stopped the bleeding in this region.

IX- Histopathological examination:

Specimens from liver and kidney tissues were fixed in 10 % buffered formalin, dehydrated in alcohol, cleared with xylene and embedded in paraffin wax. Four micron sections were cut and stained with haematoxyline and eosin (HE) **(Bancroft *et al.*, 1996).** Stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan).

I- Statistical analysis:

The data were expressed as mean ± Standard error (SE). The results were analyzed statistically using column statistics and one way ANOVA with Newman-Keuls Multiple comparison test as a post-test. These analyses were carried out using computer statistics prism 3.0 package (Graph pad software, Inc, San D.).

RESULTS

1) Carbonyl protein (CP) content:

Table (2) showed a significant elevation of hepatic CP level in *G. gerbillus* ($p<0.01$) and insignificant decrease of this level in *A. niloticus* and *R. rattus* ($p>0.05$) which had been fed with Coumatetralyl compared with control. Renal CP level increase significantly in *Gerbillus gerbillus* ($p<0.001$) and *Arvicanthis niloticus* ($p<0.05$), however, the level of CP decrease insignificantly in *Rattus rattus* ($p>0.05$) which had been fed with coumatetralyl compared with control. The present table also showed a significant elevation in erythrocyte lysate CP levels in *G. gerbillus*, *A. niloticus* and *R. rattus* ($p<0.001$) of coumatetralyl treated rats compared with control.

2) Catalase activity (CAT):

The current data obtained from **Table (3)** showed that hepatic CAT activity increased significantly in *G. gerbillus* ($p<0.001$) and insignificantly in *A. niloticus* ($p>0.05$). However, the activity of hepatic CAT decreased significantly in *R. rattus* ($p<0.001$) which had been fed with coumatetralyl compared with control rats. The present table also showed insignificant elevation in renal CAT activity in *G. gerbillus* ($p>0.05$). Nevertheless there was a significant decrease in the activity of renal CAT in *R. rattus* ($p<0.001$), and an insignificant decrease in this activity in *A. niloticus* ($p>0.05$) of rats treated with coumatetralyl compared with the control. Also, the same table showed a significant decrease in erythrocyte lysate CAT activity in *G. gerbillus*

($p<0.01$) and *A. niloticus* ($p<0.001$). Though, there was an insignificant decrease in this activity in *R. rattus* ($p>0.05$) which had been fed with coumatetralyl compared with control rats.

3) Superoxide dismutase (SOD):

Table (3) showed a significant decrease of SOD level in the liver tissues of *G. gerbillus*, *A. niloticus* and *R. rattus* ($p<0.001$) in coumatetralyl treated rats in comparison with the control rats. The present table also showed a significant decrease in renal SOD level in *G. gerbillus* and *R. rattus* ($p<0.001$). While, there was an insignificant elevation in the level of renal SOD in *A. niloticus* ($p>0.05$) which had been fed with coumatetralyl compared with control.

4) Glutathione (GSH):

The data obtained from **Table (4)** indicated that the content of hepatic GSH decreased significantly in *G. gerbillus* and *A. niloticus* ($p<0.001$). Although the content of hepatic GSH decreased in *R. rattus* but didn't reach the significant level ($p>0.05$) in coumatetralyl treated rats in comparison with the control rats. The same table also represented that renal GSH content elevated significantly in *G. gerbillus* ($p<0.001$) and insignificantly in *A. niloticus* ($p>0.05$). In addition, the content of renal GSH decrease significantly in *R. rattus* ($p<0.001$) which had been fed with coumatetralyl compared with control rats. Moreover, erythrocyte lysate GSH content reduced significantly in *G. gerbillus* ($p<0.001$) and *A. niloticus* ($p<0.05$) but this reduction was insignificantly in *R. rattus* ($p>0.05$) in coumatetralyl treated rats compared with control rats.

5) Clotting parameters:

Table (5) showed a significant elevation in plasma prothrombin time and INR time in *G. gerbillus* ($p<0.001$), ($p<0.05$) respectively, *A. niloticus*, and *R. rattus* ($p<0.001$) treated with coumatetralyl in comparison with control rats. All species showed insignificant alteration in FDPs which it was less than 5 in control rats and rats had been fed with coumatetralyl. The present table also indicated a significant increase in blood clotting

time in *G. gerbillus*, *A. niloticus* and *R. rattus* ($p<0.001$) treated with coumatetralyl compared with control rats. Furthermore, **table (5)** represented a significant increase in plasma bleeding time in *G. gerbillus*, *R. rattus* ($p<0.001$) and *A. niloticus* ($p<0.05$) of coumatetralyl treated rats compared with control.

6) Histopathological results:

Histopathological findings of the kidney and liver were evaluated by light microscopy. Incidence of the lesions in the studied groups is summarized in **Table (6).** Examination of the kidney of *Gerbillus gerbillus* of control rats showing normal histological architecture **(Fig. 1A).** Histopathological examination of HE-stained kidney sections revealed that the treatment of rats with coumatetralyl showed vascular changes were in the form of interstitial edema, congestion of the blood vessel **(Fig. 1B)** and swelling of the glomeruli as a result of congestion **(Fig. 1C).** Periglomerular mononuclear cell infiltrations in the form of lymphocytes were also seen. Examination of the kidney of *Arvicanthis niloticus* revealed normal histological architecture in control group **(Fig. 1D).** The histopathology of the kidney of coumatetralyl treated rats showing marked glomerular swelling and dilatation of Bowman's spaces due to edema. In addition, heavy infiltration with mononuclear cells in the intersitium were noticed **(Fig. 1E).** Kidney of *Rattus rattus* of the control group showed normal histological architecture **(Fig. 1F).** While, kidney of coumatetralyl treated rats showed glomerular swelling and proliferation of mesangial cells.

Examination of the liver of *Gerbillus gerbillus* of control rats showed normal histological architecture **(Fig. 2A).** Histopathological examination of HE-stained liver sections revealed that the treatment of rats with coumatetralyl showed vascular changes were in the form of vascular and cellular changes. In the form of congestion of the central vein **(Fig. 2B).** The hepatocellular changes were in the form of multiple focal areas of necrosis infiltrated with mononuclear cells **(Fig. 2C).** The liver of *Arvicanthis niloticus* of control rats showed normal

histological architecture **(Fig. 2D).** Examination of the liver of coumatetralyl treated rats revealed pronounced vascular changes in the form of congestion of the central veins and severe dilation of veins (Telangectasis) **(Fig. 2E).** Liver of *Rattus rattus* of control rats revealed normal histological architecture **(Fig. 2F).** The histopathological examination of the liver of coumatetralyl treated rats showed vascular changes in the form of congestion of the central vein and hepatocellular changes in the form of vacuolar degeneration of the hepatocytes **(Fig. 2G).**

DISCUSSION

Anticoagulant rodenticides are the greatest ordinarily used pesticides to control harmful rodent populations **(Albert *et al.*, 2009).** Coumarins such as warfarin, dicoumarol, and Coumatetralyl, and indandiones such as valone and pindone belong to the first generation. **(Park *et al.*, 2011).** Depending on their chemical structure, rodenticides are subdivided into first generation (couma-chlor, coumafuryl, coumatetralyl and warfarin) and second generation (brodifacoum, bromadiolone, difenacoum, difethialone and floco-umafen) compounds **(Valchev *et al.*, 2008)**. coumatetralyl is an anticoagulant of the 4-hydroxycoumarin vitamin K antagonist type used as a rodenticide **(Reigart and Roberts, 2013).** It is something of an exception, being generally more potent than the others and effective against warfarin-resistant Norway rats; its applicability is probably more similar to that of the indandione and second generation anticoagulants **(Greaves, 1989).**

Some rat populations have evolved resistance to some of toxic anticoagulants such as warfarin, bromadiolone and difenacoum **(Lund, 1988).** The percentage of coumatetralyl in wheat bait was 0.0375% in the current study and is similar to that performed by **Mikhail and Hassan (2016).** In the present study, the LD_{50} (delayed 96 hour) of coumatetralyl of the three dominant rodents species: *Rattus rattus*, *Arvicanthis niloticus* and *Gerbillus gerbillus* were 39.34, 35.29 and 42.84 mg /Kg b.w. respectively. On the other hand, **Mikhail and Hassan (2016)** recorded that the

required dose consumed of coumatetralyl (in bait consumption: 25.4 g) of 100 % mortality with zero resistance after 9.15 days, was 76.4 mg/kg for *Rattus rattus* (of both sexes). In addition, In *Rattus norvegicus*, the required dose consumed of coumatetralyl (in bait consumption: 36.15 g) of 100 % mortality with zero resistance after 5.5 days, was 53.3 mg/kg of both sexes **(Mikhail and Hassan, 2016).** The oral ½ LD_{50} value of coumatetralyl in the most sensitive species (rat) tested is 30/15 mg/kg b.w. for males and females, respectively. Other mammalian species (mouse, dog cat, guinea pig, and rabbit) are less susceptible to single oral doses of coumatetralyl **(Bomann, 1992).**

The present result showed that *Gerbillus g*erbillus with 80 g b.w. and bait consumption (b.c.) of 8 g, the LD_{50} of COM was 42.84 mg/kg, *Arvicanthis n*iloticus with 170 g b.w. and b.c. of 16 g, the LD_{50} of COM was 35.29g/kg b.w., while *Rattus rattus* with 110 g b.w. and b.c. of 12g, the LD_{50} of COM was 39.34 mg/kg. This result may let us to arrange these species from high to low doses required for LD_{50} of COM as follow: *Gerbillus g*. followed by *Rattus r.*, followed by *Arvicanthis n*. The data obtained in the current study reveals that the greatest LD_{50} of COM was in Gerbillus g. in spite of smallest average b.w., while the lesser LD50 of COM was in *Arvicanthis n.* in spite of its bigger average b.w. Consequently, it could suggest that the LD50 of COM for the wild rats was species-dependent not body weight dependent. The present study determined the LD50 of COM which kills half number of rat species with different dose and wheat baits after 4 days in contrary to that showed by **Mikhail and Hassan (2016)** who used doses of LD50 COM kill half number of rats through 6 days.

Warfarin intake (0.35 mg/l and 3.5 mg/l for 30 days in rats) resulted in histologically evident tissue damage, leukocyte infiltration and intestinal inflammation increases in myeloperoxidase activity, malondialdehyde content, superoxide dismutase and catalase activity **(Mirkov *et al.*, 2016).** In the present study, there are significant depletion of CAT activity in

erythrocytic lysate in *Gerbillus gerbillus* and *Arvicanthis niloticus*, in both liver and kidney of *Rattus rattus* in response to ¼ LD_{50} comparing to control. This depletion in CAT activity may associated with disturbances in the activity of G6PD found in all tissue cells. Supporting this interpretation, it is found that the impaired catalase activity underlies the enhanced oxidant sensitivity of G6PD-deficient erythrocytes and elucidates the importance of NADPH in the maintenance of normal catalase activity **(Scott *et al*., 1991).**

In the present study, ¼LD_{50} of COM significantly decreased SOD activity in liver of all studied rat species *Gerbillus Gerbillus*, *Arvicanthis niloticus* and *Rattus rattus*, while in kidney, the SOD depletion did not appear except of *Rattus rattus*. Consistently, bromadiolone exposure reduced SOD in erythrocyte and liver of birds **(Sodhi *et al*., 2017).**

In the present study, ¼ LD_{50} of COM significantly decreased GSH level in erythrocytic lysate in *Gerbillus gerbillus* and *Arvicanthis niloticus* probably due to the depletion of NADPH that required to regenerate GSH during counteracting the overproduction of ROS in response to COM treatment in RBCs which may suffer from filling of H_2O_2.

Almroth *et al*. (2005) found that the formation of carbonyl derivatives is non-reversible, causing conformational changes, decreased catalytic activity in enzymes and ultimately resulting in breakdown of proteins by proteases due to increased susceptibility. Moreover, oxidative modification of proteins may lead to the structural alteration and functional inactivation of many enzyme **(Stadtman *et al*.. 1991)**.

In the present study, there were significant elevations of CP in erthyrocytic hemolysate of all treated studied rat species, while led to significant rise in renal CP of *Gerbillus gerbillus* and *Arvicanthis niloticus*. Nevertheless, pronounced rise in the liver CP of *Gerbillus gerbillus* in response to ¼COM. This marked rise in CP may participate in the decrease of hepatic SOD activity observed in the present study rather than its role in counteracting

overproduction of ROS in response to ¼ LD_{50} of COM in liver of *Gerbillus Gerbillus* and *Arvicanthis niloticus* and in kidney of *Rattus rattus*. Similarly, liver CP levels were significantly elevated in insecticide diazinon-treated mice **(El-Shenawy *et al*., 2010).** In addition the activity of CAT was significantly decreased in some species in response to ¼ LD_{50} of COM possibly associated with elevated CAR. PR level in the present study as well as its role in consumption of excess ROS.

INR as an indicator of re-establishment of normal hemostasis depends not only on the clearance of the vitamin K antagonists but also on the capacity of the liver to synthesize the coagulation factors II, VII, IX and X. The latter is also contingent on the availability of vitamin K **(Schulman *et al*., 2008).** The present study showed significant increases in INR, PTT, clotting time and bleeding time in all tested species of rodents: in response to coumatetralyl supplementation. Consequently, these results may be essentially associated with liver damage where some clotting factors are synthesized.

It is well known that fibrin degradation products (FDPs), known as fibrin split products, are components of the blood produced by clot degeneration **(Gaffney *et al*., 1995). Cade *et al.* (1975)** suggested that under these experimental conditions the elevated levels of FDP in pulmonary embolism are derived mainly from lysis of fibrin deposited after embolization rather than from lysis of the original embolus. In the present study, the FDP levels were < 5 in both control and COM-treated groups. Similarly, in dog administered coumatetralyl, the FDP remained within the normal range **(Park *et al*., 2011).**

In the present study, the histopathological examination of HE-stained kidney sections revealed that the treatment of the three dominant species with coumatetralyl showing vascular changes. These changes were congestion, glomerular swelling and interstitial edema. Similar results was reported by **Murphy (2002)** and **Radi and Thompson (2004)** who stated that the derivatives of coumarin including coumatetralyl provoke severe injury to

vascular permeability, resulting in massive hemorrhages and the rapid death of rodents. The kidney tissues of the studied rats also showed signs of inflammation as proliferation of mesangial cells and periglomerular inflammatory cellular reactions. Similarly, **Buckley (1989)** reported that in *Mus Musculus* consumed 12.526 mg/kg bromadiolone after 48 h led to necrosis, degeneration and accumulation of toxic metabolic debris in the renal glomerular and tubular cortex region.

In the current study, the histopathological examination of HE-stained liver sections revealed that the treatment of the three dominant species with coumatetralyl showing vascular changes and cellular changes. These changes were in the form of congestion, degeneration, necrosis and inflammatory cellular reactions. These results were in harmony with the studies of **Revathi and Yogananda (2006)** who demonstrated that the liver of *Mus Musculus* intoxicated with bromadiolone after 48 h there was a clear multifocal cytoplasmic vacuolations, necrosis and accumulation of toxic debris.

In conclusion, The ¼ LD_{50} of COM elevates oxidative stress parameters, decreasing antioxidants in erythrocytic lysate, kidney and liver tissues, affect kidney and liver function in addition it exerts deleterious effect on histological structure of liver and kidney in the three dominant species of rodents with different extents. The present results suggested that the effects of ¼ LD_{50} of COM on the three dominant species were very toxic as well as it has a potent ability to reduce these rodents by lower cost.

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Table (2): Effect of ¼ LD_{50} coumatetralyl on carbonyl protein (CP) in hepatic & renal tissues and erythrocytic lysate of the three dominant wild rat species.

	Gerbillus gerbillus			*Arvicanthis niloticus*			*Rattus rattus*		
Groups	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**
Hepatic CP nmol/mg protein	15.53±0.94	22.82±3.6**	46.94	20.07±1.11	18.61±0.79	-7.28	20.62± 1.80	17.01± 1.10	-17.51
Renal CP nmol/mg protein	10.93±0.37	14.27±0.15***	30.56	15.38±0.27	16.33±0.09*	6.18	14.76± 0.19	14.07± 0.45	67
Erthyrocytic lysate CP nmol/mg Hb	9.318±0.52	19.63±1.59***	110.67	9.536±0.69	19.39±1.55***	103.33	12.71±0.20	22.54±0.92***	77.341

Data are present as means ± SE. Number of rats (n) =10. * means $P < 0.05$, ** means $P < 0.01$ and *** means $P < 0.001$.

Table (3): Effect of ¼ LD_{50} Coumatetralyl on catalase (CAT) and superoxide dismutase (SOD) activities in hepatic renal tissues and erythrocytic lysate in different dominant wild rat species.

Species	***Gerbillus gerbillus***			***Arvicanthis niloticus***			***Rattus rattus***		
Parameters / Groups	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**
Hepatic CAT activity (U/min/mg protein)	12.91± 0.64	17.32±1.09***	34.16	13.79±0.33	15.51±0.43	12.47	23.56± 0.90	11.05± 0.57***	-53.098
Renal CAT activity (U/min/mg protein)	33.70± 1.77	38.77±2.13	15.05	41.87± 1.99	40.59±1.76	-3.06	53.01±2.40	28.79± 1.33***	-45.689
CAT activity in Erthyrocytic lysat (U/min/mg Hb)	30.51±0.61	24.71±1.09**	-19.01	26.98± 1.77	14.05±0.84***	-47.92	18.75±1.38	16.42± 0.64	-12.426
Hepatic SOD (U/min/mg protein)	3.708± 0.23	0.378±0.07***	-89.80	4.855± 0.03	2.763±0.15***	-43.09	4.396± 0.35	0.881± 0.14***	-79.964
Renal SOD (U/min/mg protein)	0.250± 0.02	0.066±0.02***	-73.55	0.589± 0.05	0.63± 0.05	7.07	2.104± 0.14	0.429± 0.09***	-79.610

Table (4): Effect of ¼ LD_{50} coumatetralyl on GSH content in hepatic & renal tissues and erythrocytic lysate in the three dominant wild rat species.

	Gerbillus gerbillus			***Arvicanthis niloticus***			***Rattus rattus***		
Parameter / Groups	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**
Hepatic GSH (ng/mg protein)	53.21±1.24	40.88±2.36***	-23.17	68.54±1.46	36.39±1.70***	-46.91	41.14± 1.641	37.37±0.8308	-9.16
Renal GSH (ng/mg protein)	23.16±0.85	42.49±1.97***	83.46	25.71±1.03	23.05±1.407	-10.35	48.57±1.035	30.96±1.144***	-36.26
GSH in erthyrocytic lysat (ng/mg Hb)	38.29±2.05	24.67±1.6***	-35.57	24.62± 1.50	18.42±1.335*	-25.18	19.74± 0.93	19.55± 0.9862	-0.96

Data are present as means ± SE. Number of rats (n) =10. * means $P < 0.05$, ** means $P < 0.01$ and *** means $P < 0.001$.

Table (5): Effect of ¼ LD_{50} coumatetralyl on prothrombine time (PT), international normal ratio (INR), fibrinogen degradation product (FDP), clotting time and bleeding time in the three dominant wild rat species.

Species	***Gerbillus gerbillus***			***Arvicanthis niloticus***			***Rattus rattus***		
Parameter / Groups	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**	**Control**	**Treated**	**% of change**
PT in plasma (Sec.)	10.73±1.08	274.1±16.1***	2454.5	24.10±1.63	989.5±6.01***	4005.8	13.0±0.31	603.0±45.92***	4538.5
INR in plasma (Sec.)	0.657±0.06	103.6±4.98*	15668.6	1.83± 0.08	665.5±2.59***	36266	0.992±0.033	522.8±50.27***	53078.8
FDP	Less than 5	Less than 5		Less than 5	Less than 5		Less than 5	Less than 5	
Clotting time (min.)	0.560±0.10	5.445±0.58***	871.97	1.933±0.14	17.53±0.44***	806.88	0.898±0.14	8.382±0.43***	833.62
Bleeding time (min.)	1.467± 0.12	3.026±0.25***	106.27	3.199±0.19	3.864±0.23*	20.79	1.428±0.17	2.869±0.24***	100.91

Data are present as means ± SE. Number of rats (n) =10. * means $P < 0.05$, ** means $P < 0.01$ and *** means $P < 0.001$.

Table (6): Incidence of histopathological lesions in renal & hepatic tissues of the three dominant wild rat species.

Lesion / Groups	*Gerbillus gerbillus* treated group	*Gerbillus gerbillus* control group	*Arvicanthis niloticus* treated group	*Arvicanthis niloticus* control group	*Rattus rattus* treated group	*Rattus rattus* control group
Kidney						
Congestion	++	-	+	-	+	-
Glomerular swelling	++	-	+++	-	+	-
Mononuclear infiltration	++	-	++	-	++	-
Interstitial edema	++	-	+	-	-	-
Liver						
Congestion	+++	-	+++	-	++	-
Telangectasis	-	-	+	-	-	-
Vacuolar degeneration	-	-	-	-	++	-
Focal necrosis	++	-	-	-	-	-
Mononuclear infiltration	+++	-	+	-	+	-

– no lesions; + lesions found in 1–3 rats; ++ lesions found in 4–6 rats; +++ lesions found in 7–10 rats.

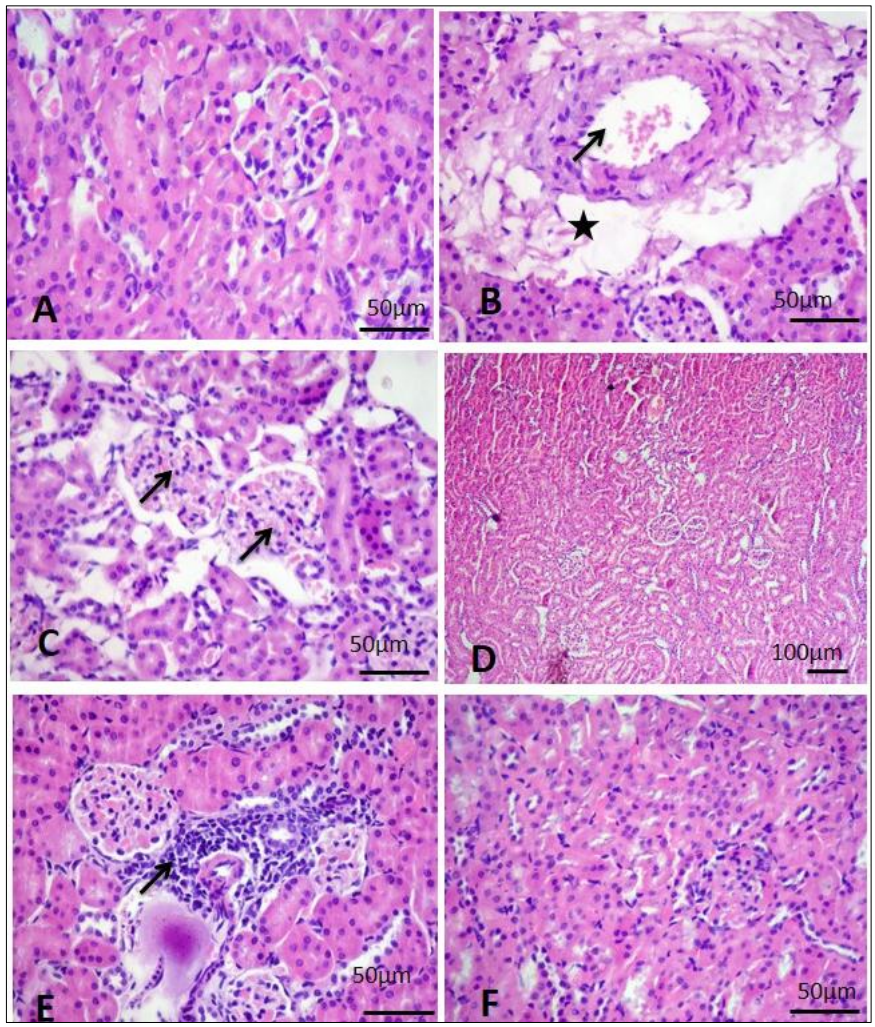

Fig. (1): Kidney of Geribellus geribullus A) Kidney of control rats showing normal histological architecture. B) Kidney of coumatetralyle treated rats showing interstitial edema (star), congestion of the blood vessel (arrow). C) Glomerular swelling (arrows). D) Kidney of Arciphenthesis niloticus, kidney of control rats showing normal histological architecture. E) Kidney of coumatetralyle treated rats showing mononuclear cell infiltration in the intersitium (arrow). F) Kidney of Rattus rattus, kidney of control rats showing normal histological architecture. HE.

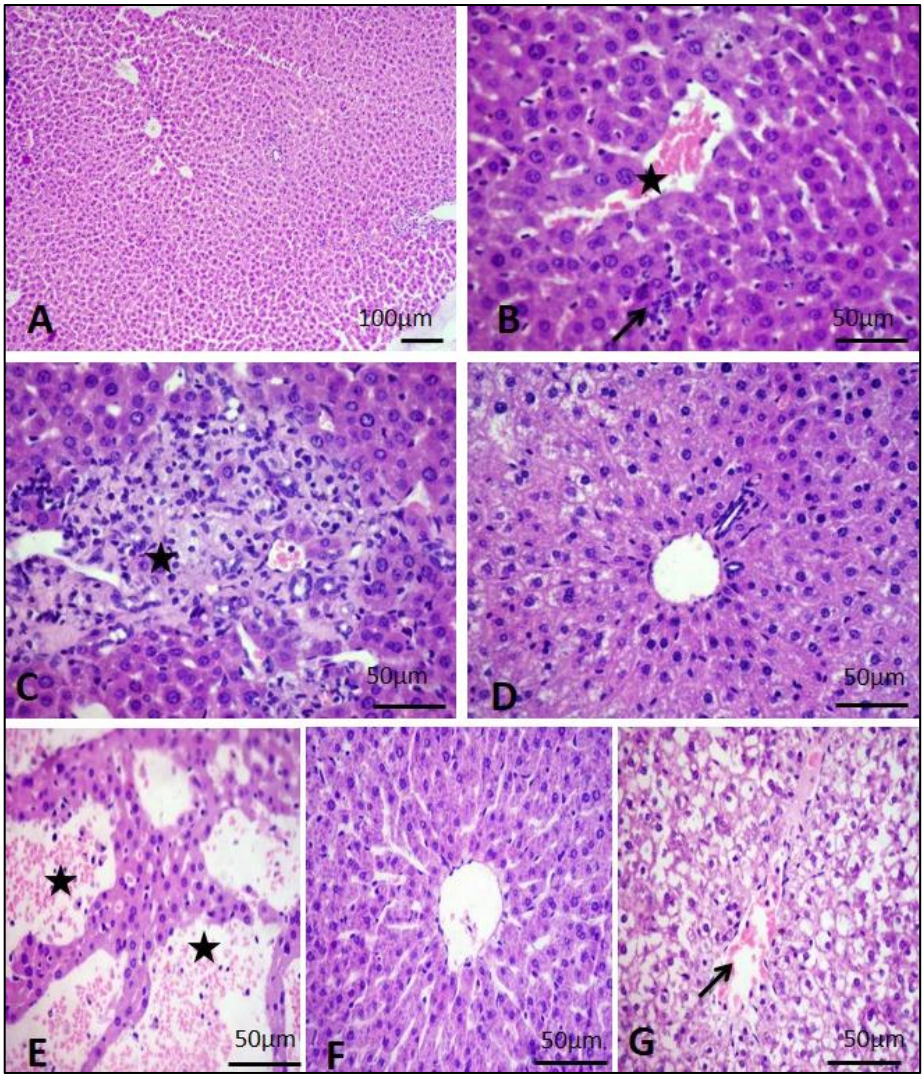

Fig. (2): Liver of Geribellus geribullus A) Liver of control rats showing normal histological architecture. B) Liver of coumatetralyle treated rats showing congestion of the central vein (star), mononuclear cell infiltration (arrow). C) Focal area of necrosis infiltrated with mononuclear cells (stars). D) Liver of control Arciphenthesis niloticus rats showing normal histological architecture. E) Liver of coumatetralyle treated rats showing evere dilation of veins (Telangectasis) (stars). F) Liver of control Rattus rattus showing normal histological architecture. G) Liver of coumatetralyle treated rats showing congestion of the central vein (arrow) and vacuolar degeneration of the hepatocytes. HE.

تعتبر القوارض ضمن معظم الافات التي تهم الزراعة المصرية. في معظم البلدان يتم استخدام الوارفارين غالبا ولكن ظهر جليا قدر من المقاومة لهذا المبيد مما اوجد مشكلة. المبيدات المسيلة للدم مثل الكوماتترااليل المثبط . وهذه الدراسة محاولة لتعيين الجرعة النصف مميتة Kلعمل فيتامين للكوماتترااليل لثلاثة فئران برية سائدة في اربع مراكز في محافظة اسيوط هي منفلوط وديروط وابنوب واسيوط. ايضا لدراسة وتفسير التاثيرات الضارة لربع الجرعة النصف مميتة للمبيد قد تم قياس الكاربونيل بروتين كمثال لنواتج الاكسدة وقياس الجلوتاثيون ونشاط الكتاليز والسوبر اكسيد ديسميوتيز كمثال لمضادات الاكسدة في الكبد والكلى والهيموليزات. والمعامل الطبيعي الدولي (P T)وايضا تم قياس زمن البروثرومبين وزمن التجلط وزمن النزفز (FDPs) ومنتج الهدم للفيبرين (INR) بالاضافة فحص الهستوباثولوجى لكل من انسجة الكبد والكلى. والنتائج اوضحت ان نصف الجرعة المميتة في الفئران التي اظهر البحث سيادتها في الاربع مراكز الدراسة وهي كالتالي: الجرذ المتسلق والجرذ النيلي 39.34,(فأر الغيط) وفأر الجربيلس الصحراوي المصري الصغير كانت ملليجرام / كيلو جرام من وزن الجسم علي الترتيب. وقد 42.84 ,35.29 أظهر البحث ارتفاع معنوي للكاربونيل بروتين وانخفاض معنوي لمضادات الاكسدة في معظم العينات المقاسة للفئران الثلاثة. أوضحت وزمن البرثرومبين وزمن التجلط INRالدراسة ايضا ارتفاع معنوي في لم تظهر تغيرا يذكر. ويمكن الاستنتاج أن ربع FDPsوزمن. ولكن الجرعة النصف مميتة للمبيد لها تأثير سام جدا وكذلك حساسية قوية علي الفئران محل الدراسة. لذلك هذه الجرعة المستخدمة اثبتت ان لها مقدرة كبيرة في تقليل هذه الافات بأقل تكلفة واقل نسبة للتلوث