RESULTS & DISCUSSION

4- RESULTS AND DISCUSSION

4-1-Feed intake:

Average feed consumption was calculated as shown in **Fig.1.** The 1st group (control) did not show any clinical manifestations through the course of the experiment.

The averages of feed consumption through the experimental period were 73.3gm/ kg. BW/day, 60gm / kg. BW/day and 42.2gm / kg. BW/day for 60 days in the 1st group (control), 2nd group (fed 3.95gm copper oxychloride fungicide/kg feed) as 1/20 LD_{50} fed 0.1184gm Cu/kg. BW/day and 3rd group (fed 11.81gm copper oxychloride fungicide/kg feed) as 1/10 LD_{50} fed 0.2368gm Cu / kg. BW/day, respectively in **Table 1.**

The obtained results from this study showed that, the copper oxycloride fungicide lead to reduce feed consumption (by about 18.05 % and 42.4 % in both 2nd and 3rd groups, respectively in comparison with control group). These reductions may be due to the copper compound which leads to loss of appetite and general depression. These results are in agreement with **Haywood (1979 &1985)** in rats and **Abdel-Hafez (1995)** in rabbits. These authors reported symptoms of copper toxicity in different species of animals were anorexia, thirsty, general depression and loss of appetite.

4-2-Haematological assessment:

4-2-1-Hemoglobin (Hb):

The least square mean values of hemoglobin concentration (Hb) in New Zealand White rabbits (NZW) under different

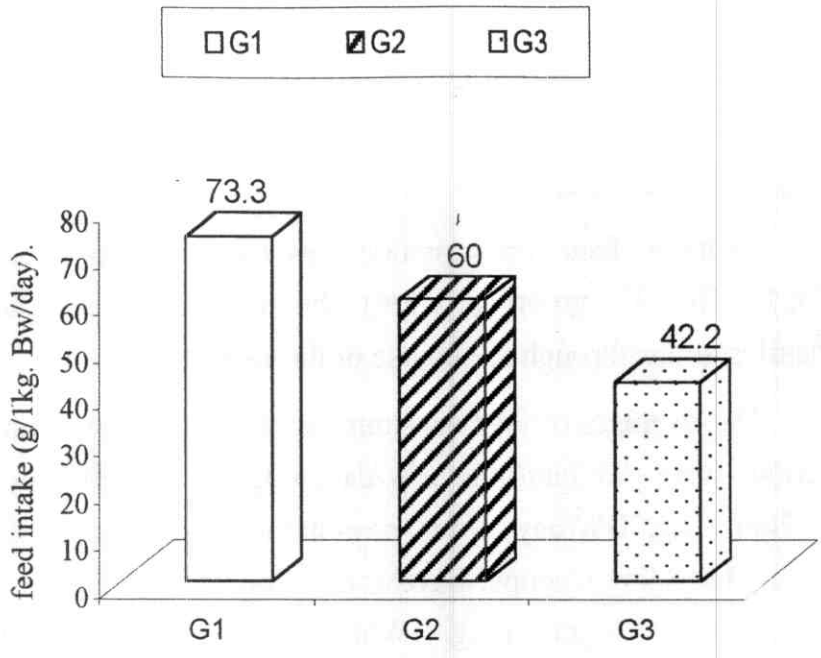

Fig. 1: Effect of copper oxychloride fungicde intoxication on feed consumption in rabbits.

Table 1: Average of copper oxychloride fungicide supplement/kg feed, total copper in feed, copper intake and feed consumption.

Groups	Average of fungicide g/kg adds in feed	Total copper g/kg. feed 50% Cu	Average of copper taken g/kg. BW/day	Average of feed consumption g/kg. BW/day
1st group	0.0	0.01	0.0007	73.3
2nd group	3.950	1.995	0.1184	60
3rd group	11.810	5.925	0.2368	42.2

1st group = Control
2nd group = (0.1184 gm Cu /kg.BW/day)
3rd group = (0.2368 gm Cu/kg.BW/day)

RESULTS AND DISCUSSION

treatment are presented in **Table 2** were 11.85±2.25, 10.49±2.25 and 8.73±2.25 gm/dl for 1st group (control), 2nd group (treated with 1/20 LD_{50} fungicide as 0.1184gm Cu/kg.BW/day) and 3rd group (treated with 1/10 LD_{50} fungicide as 0.2368gm Cu/kg.BW/day) during the experimental periods, respectively. From the results presented, the highest value in Hb concentration in 1st group which was 11.85±2.25 gm/dl while the lowest value of Hb concentration was 8.73±2.25 gm/dl in 3rd group. From the results obtained in **Table 3** and **Fig.2**, it can be observed that, there were significant differences ($P<0.05$) in Hb concentration between groups, due to the effect of treatment. The obtained results showed that Hb concentration at different periods 20 days, 40 days and 60 days feeding were 10.40±1.97, 10.04±2.21 and 9.47±2.55 gm/dl respectively, the lowest mean value of Hb concentration after 60 days feeding of treatment. The obtained results showed that Hb concentration was 8.12±4.41 gm/dl decreased when rabbits fed on 0.2368 gm Cu/kg.BW/day for 60 days (3rd group).

These results are in agreement with **Hill (1977), Dzhurov (1979)** and **Patel *et al.* (1998)** in different animals, they found that, the high copper diets given to pig and sheep lead to decreasing of Hb concentration and **Abdel-Hafez (1995)** who showed that, the Hb concentration decrease when copper diets given to rabbits for 60 days.

The normal average of Hb concentration reported in rabbits was 12-12.5 gm/dl and 13.4 gm /dl by **Melby and Altman (1976)** and 10.00-15.50 gm/dl by **Harkness and Wagner (1983).**

Table 2: Least square means and standard error for copper intoxication on Hb, PCV %, RBC_s and WBC_scount in NZW rabbits.

Items	Hb (gm/dl)	PCV (%)	RBCs $NX10^6/mm^3$	WBCs $NX10^3/mm^3$
Main effects:				
Groups (G):				
1st Group (G_1)	11.85 ± 2.25^{a}	39.16 ± 0.56^{a}	5.88 ± 0.05^{a}	7.21 ± 0.09^{a}
2nd Group (G_2)	10.49 ± 2.25^{a}	32.93 ± 0.56^{b}	4.05 ± 0.05^{b}	6.57 ± 0.09^{b}
3rd Group (G_3)	8.73 ± 2.25^{b}	29.03 ± 0.56^{c}	3.48 ± 0.05^{c}	4.44 ± 0.09^{c}
Periods (P):				
20 Days	10.40 ± 1.97^{a}	34.14 ± 0.49^{a}	4.64 ± 0.09^{a}	6.20 ± 0.08^{a}
40 Days	10.04 ± 2.21^{a}	34.08 ± 0.55^{a}	4.51 ± 0.05^{a}	6.16 ± 0.08^{a}
60 Days	9.74 ± 2.55^{b}	32.90 ± 0.64^{a}	4.25 ± 0.06^{b}	5.86 ± 0.10^{b}
Interaction:				
G_1 x 20	11.78 ± 3.42^{ab}	38.22 ± 0.86^{a}	5.71 ± 0.08^{a}	7.00 ± 0.13^{abc}
G_1 x 40	11.98 ± 3.82^{ab}	38.83 ± 0.96^{a}	5.96 ± 0.09^{a}	7.19 ± 0.14^{ab}
G_1 x 60	11.78 ± 4.41^{ab}	40.42 ± 1.10^{s}	5.96 ± 0.11^{a}	7.81 ± 0.17^{a}
G_2 x 20	11.06 ± 3.42^{a}	33.57 ± 0.86^{b}	4.34 ± 0.08^{b}	6.81 ± 0.13^{bc}
G_2 x 40	10.10 ± 3.82^{ab}	33.29 ± 0.96^{b}	3.98 ± 0.09^{c}	6.70 ± 0.14^{c}
G_2 x 60	9.33 ± 4.41^{ab}	31.93 ± 1.10^{bc}	3.83 ± 0.11^{cd}	6.19 ± 0.17^{d}
G_3 x 20	9.31 ± 3.42^{b}	30.63 ± 0.86^{bc}	3.88 ± 0.08^{c}	4.78 ± 0.13^{e}
G_3 x 40	8.77 ± 3.82^{b}	30.12 ± 0.96^{c}	3.58 ± 0.09^{d}	4.60 ± 0.14^{e}
G_3 x 60	8.12 ± 4.41^{b}	26.35 ± 1.10^{d}	2.96 ± 0.11^{e}	3.95 ± 0.17^{e}

Means with the same letter within each column in each factor are non-significant.
G_1 =Control; G_2 =fed on 0.1184 gm Cu/kg. BW/day; G_3 =fed on 0.2368 gm Cu/kg. BW/day.

Table 3: F-ratio of analysis of variance for the effect of copper intoxication on Hb, PCV%, RBC_s and WBC_s count in NZW rabbits.

S.O.V.	df.	Hb (gm/dl)	PCV (%)	RBCs $NX10^6/mm^3$	WBCs $NX10^3/mm^3$
Group (G)	2	1.15^{*}	81.95^{***}	534.59^{***}	288.62^{***}
Period (P)	2	1.00^{*}	1.36	13.10^{***}	4.26^{***}
G x P	4	0.96	2.87	10.86^{***}	5.21^{***}
Error df	63				
Error MS		116.70	7.31	0.07	0.17

* = Significant ($P < 0.05$).
***= Highly Significant ($P < 0.001$).

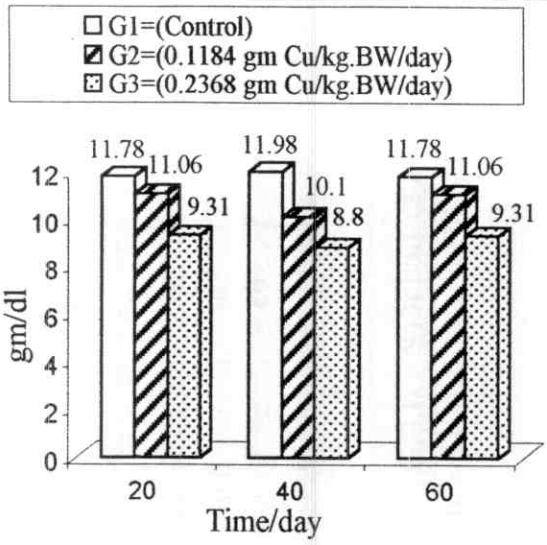

Fig.2: Effect of copper oxychloride fungicide intoxication on hemoglobin(Hb).

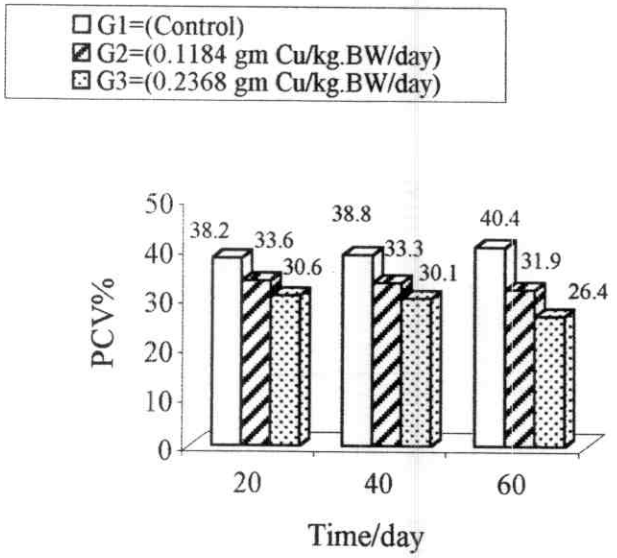

Fig.3: Effect of copper oxychloride fungicide on PCV%.

4-2-2-Packed cell volume (PCV %):

The least square means of packed cell volume (PCV%) of NZW rabbits shown in **Table 2** were 39.16±0.56, 32.93±0.56 and 29.03±0.56 for 1st, 2nd and 3rd groups, respectively. The high value in PCV % in 1st group (control) was 39.016±0.56 while the lowest value was 29.03±0.56 in 3rd group (fed on 0.2368 gm cu/kg. BW/day). From the results presented in **Table 3** and **Fig. 3,** it can be observed that, there were highly significantly differences (P<0.001) in PCV% between groups.

The least square means of PCV% in different periods 20 days, 40 days and 60 days were 34.14±0.49, 34.08±0.55 and 32.9±0.64 % respectively, this results were non-significantly between periods. The results between groups at different periods were 38.22±0.86, 33.57±0.86 and 30.63±0.86 for 1st , 2nd and 3rd groups at 20 days, respectively, the means of PCV% at 40 days were 38.83±0.96, 33.29±0.96 and 30.12±0.96 in the same groups respectively and there were 40.42±1.10, 31.93±1.10 and 26.35±1.10 for 1st, 2nd and 3rd groups respectively.

From the obtained results it could be showed that, PCV% was decreased when rabbits fed of 0.2368 gm Cu/kg.BW/day for 60 days. These results are in agreement with **Ecckert *et al.* (1999)** who reported that, ewes fed copper diets for 73 days had lower PCV%; **Adams *et al.* (1977) and Solaiman *et al.* (2001).** in goats, while **Schalm (1965)** reported that, the normal average of PCV% in rabbits was 36-38 % and 39 % by **Melby & Altman (1976)** and 36-48 % by **Harkness & Wagner (1983) and Adams *et al.* (1977).**

4-2-3-Erythrocytes count (RBC_S):

The average means of red blood cells counts (RBC_S) of NZW rabbits under different treatment are presented in **Table 2** were 5.88±0.05, 4.05±0.05 and 3.48±0.05 millions/mm^3 for 1st group (control), 2nd group (treated with 0.1184 gm Cu/kg.BW/day) and 3rd group (treated with 0.2368 gm Cu/kg.BW/day) throughout experimental periods, respectively. From the results presented in **Tables 2 & 3** and **Fig. 4**, it can be observed that, there were decreased highly significantly differences ($P<0.001$) in RBC_S due to the effect of treatment. From the obtained results of interaction between groups and periods, the highest mean value was 5.96±0.09 millions/mm^3 in RBC_S in 1st group for 40 days, while the lowest mean value of RBC_S was 2.96±0.11 millions/mm^3 for 60 days in 3rd group, the obtained results from interaction between groups and periods were decreased highly significantly ($P<0.001$). These results are in agreement with **Patel *et al.* (1998)** who reported that, the high copper diets given to rats decrease RBC_S counts for 60 days post treatment with copper. And with, **Paulo Cesar *et al.* (1998)** who reported that, in ewes fed on high level copper caused mild anaemia revealed by decreasing of RBC_s count. In the other hand **Schalm (1965)** reported that, the normal averages of RBC_s count in blood of rabbits were among 5-6 millions/mm^3 ; about 6.2 millions/mm^3 by **Melby & Altman (1976)** and from 4 to 7 millions/mm^3 by **Harkness & Wagner (1983).**

4-2-4-Total leucocytic count (WBC_S):

The mean values of total blood corpuscle's count (WBC_S) as shown in Table 2 were 7.21±0.09, 6.57±0.09 and 4.44±0.09

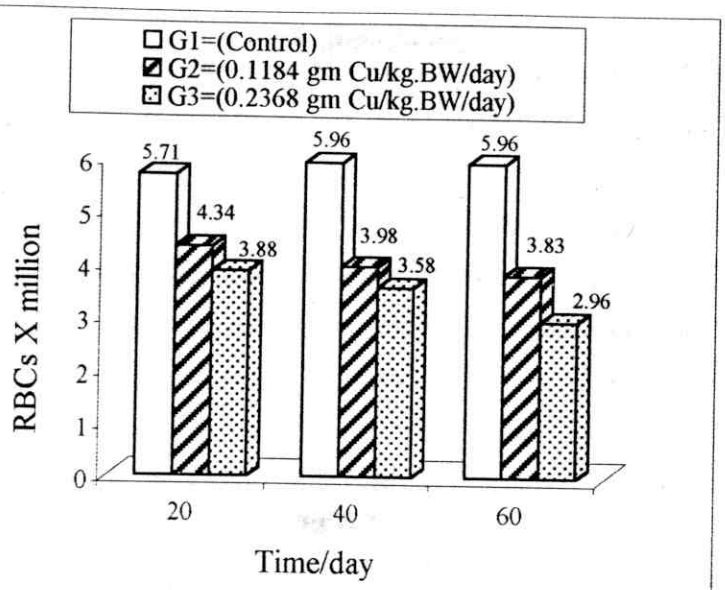

Fig.4: Effect of copper oxychloride fungicide intoxication on RBCs count.

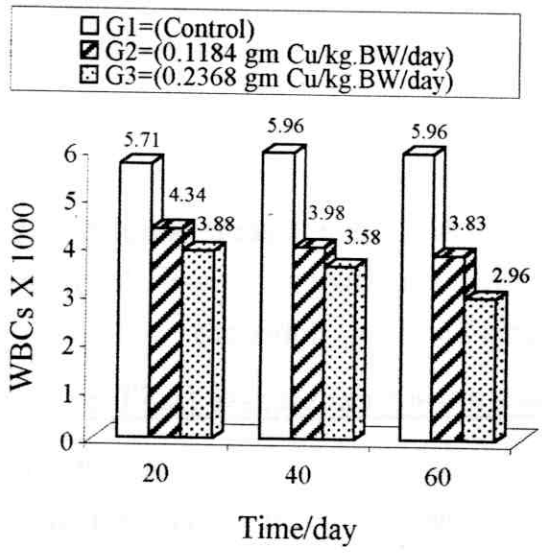

Fig.5: Effect of copper oxychloride fungicide intoxication on WBCs count.

thousand/mm^3 for 1st group (control), 2nd group and 3rd group, respectively. The means of WBC$_s$ at different periods 20, 40 and 60 days were 6.20±0.08, 6.20±0.80 and 5.86±0.10 thousand/mm^3, respectively. The obtained results showed **Tables 2&3** and **Fig. 5**, it can be observed that, the highest least square mean of interaction between groups and periods was 7.81±0.17 thousand/mm^3 in 1st group (control) for 60 days, while the lowest mean of WBC$_s$ was 3.95±0.17 thousand/mm^3 in 3rd group (fed on 0.2368 gm cu/kg.BW/day) for 60 days. From the results presented in **Tables 2&3** and **Fig. 5,** it can be observed that, there were decreased significantly (P<0.001) in WBC$_s$ count, due to the effect of treatment. The present results are in disagreement with **Solaiman *et al.* (2001)** who reported that WBC$_s$ count was increased when goat animals received oral doses of copper sulfate for 144 days and **Abdel-Hafez (1995)** who reported that, the total WBC$_s$ count increase highly significantly in NZW rabbits when exposed to copper oxychloride fungicide.

4-3-Biochemical blood assessment:

The level of serum enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determine for liver functions, and determine the level of creatinine and urea for kidney functions.

4-3-1-Aspartate aminotransferase (AST):

The obtained results revealed that, the level of AST enzymes in NZW rabbits shown in **Table 4** were 18.08±0.41, 49.16±0.41 and 64.27±0.41 u/1 for 1st group (control), 2nd group (fed on 0.1184 gm Cu/kg. BW/day) and 3rd group (fed on 0.2368 gm Cu/kg. BW/day) for 60 days, respectively. The least square

Table 4: Least square means and standard error for copper intoxication on AST, ALT, Creatinine and Urea in NZW rabbits.

Items	AST (U/1)	ALT (U/1)	Creatinine (mg/dl)	Urea (mg/dl)
Main effects:				
Groups (G):				
1st Group	18.08±0.41^{c}	16.76±0.61^{c}	0.15±0.01^{c}	31.33±1.53^{a}
2nd Group	49.16±0.41^{b}	45.72±0.61^{b}	0.36±0.01^{b}	29.75±1.53^{a}
3rd Group	64.27±0.41^{a}	81.04±0.61^{a}	0.55±0.01^{a}	28.60±1.53^{a}
Periods (P):				
20 Days	44.38±0.36^{b}	40.79±0.54^{c}	0.31±0.01^{c}	30.36±1.34^{ab}
40 Days	45.68±0.41^{a}	48.28±0.60^{b}	0.34±0.01^{b}	26.93±1.49^{b}
60 Days	41.44±0.46^{c}	54.46±0.69^{a}	0.40±0.01^{a}	32.40±1.72^{a}
Interaction:				
G_1 x 20	16.68±0.62^{h}	16.48±0.93^{f}	0.15±0.01^{f}	28.58±2.31^{ab}
G_1 x 40	18.30±0.70^{gh}	16.76±1.04^{f}	0.14±0.01^{f}	31.53±2.59^{ab}
G_1 x 60	19.27±0.80^{g}	17.05±1.20^{f}	0.16±0.01^{f}	33.88±2.99^{a}
G_2 x 20	52.51±0.62^{d}	32.30±0.93^{e}	0.32±0.01^{e}	33.78±2.31^{a}
G_2 x 40	49.38±0.70^{e}	43.53±1.04^{d}	0.34±0.01^{e}	25.87±2.59^{ab}
G_2 x 60	45.60±0.80^{f}	61.35±1.20^{c}	0.42±0.01^{d}	29.61±2.99^{ab}
G_3 x 20	63.96±0.62^{b}	73.58±0.93^{b}	0.47±0.01^{c}	28.71±2.31^{ab}
G_3 x 40	69.37±0.70^{a}	84.60±1.04^{a}	0.55±0.01^{b}	23.39±2.59^{b}
G_3 x 60	69.47±0.80^{c}	84.99±1.20^{a}	0.63±0.01^{a}	33.71±2.99^{a}

Means with the same letter within each column in each factor are non-significantly different ($P < 0.05$).
G_1= Control; G_2= fed on 0.1184 gm Cu/kg. BW/day; G_3 =fed on 0.2368 gm Cu/kg. BW/day.

means of AST enzymes in different periods 20 days, 40 days and 60 days were 44.38±0.36, 45.68±0.41 and 41.44±0.46 u/1, respectively. From the results presented in **Tables 4 &5** and **Fig. 6**, it can be observed that, there were increased significantly ($P<0.001$) in ASAT, due to the effect of copper. From the results of interactions between groups and periods, showed that, the highest value in AST enzyme was 69.47±0.80 u/1 in 3rd group (treated with high fungicide level) for 60 days, while the lowest value in ASAT enzyme was 19.27±0.80 u/1 in control group after 60 days from feeding. These results of interactions between groups and periods were increased highly significantly ($P<0.001$).

These results are in agreement with **Naoki *et al.* (1995)** who reported that, liver enzymes activities used for assessment of hepatic function increased significantly with the increase of copper concentration in diet rats and with **Blood & Henderson (1976) and Hidiroglov *et al.* (1984)**, who reported that liver enzymes increased in chronic copper poisoning of sheep. **Abdel-Hafez (1995)** showed that, AST enzyme increase in serum rabbits when fed on high level copper for 60 days.

4-3-2-Alanine aminotransferase (ALT):

The least square means of level serum ALT enzymes in NZW rabbits were 16.76±0.61, 45.72±0.61 and 81.04±0.61 u/1 for 1st group (control), 2nd group and 3rd group for 60 days, respectively **Table 4**. The obtained results for different periods 20, 40 and 60 days were 40.79±0.54, 48.28±0.60 and 54.46±0.69 u/1 in ALT levels, respectively.

Table 5: F-ratio of analysis of variance for the effect of copper intoxication on AST, ALT, Creatinine and Urea in NZW rabbits.

S.O.V.	df.	AST (U/1)	ALT (U/1)	Creatinine (mg/dl)	Urea (mg/dl)
Group (G)	2	3297.50^{***}	2757.03^{***}	979.33^{***}	0.81
Period (P)	2	24.43^{***}	127.02^{***}	46.19^{***}	3.06
G x P	4	23.51^{***}	49.18^{***}	13.16^{***}	2.02
Error df	63				
Error MS		3.86	8.64	0.001	53.52

***= Highly significant ($P < 0.001$).

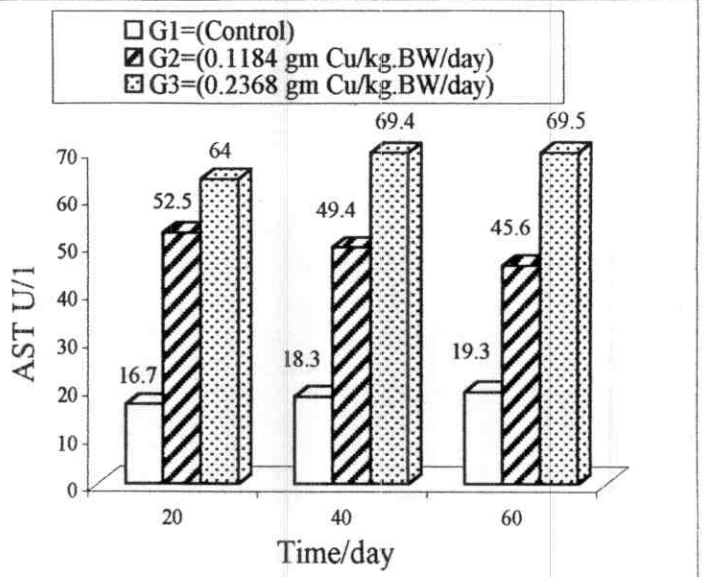

Fig.6: Effect of copper oxychloride fungicide intoxication on AST enzyme.

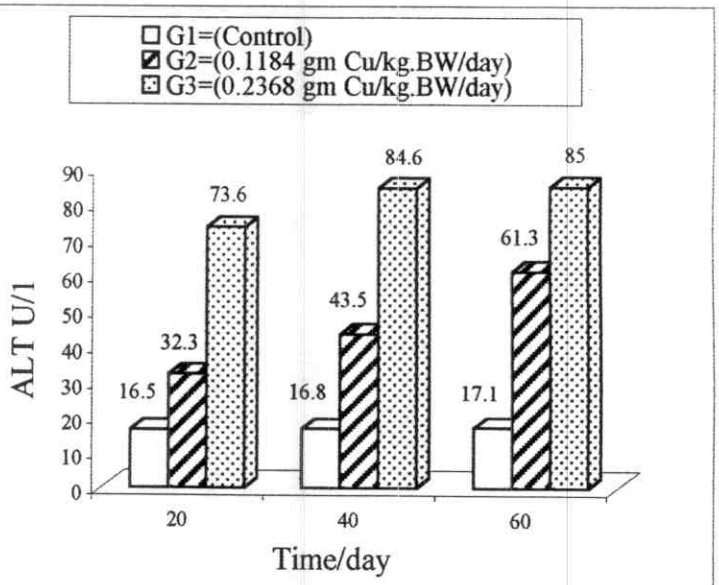

Fig.7: Effect of copper oxychloride fungicide intoxication on ALT enzyme.

From the obtained results showed that, the mean level of ALT enzyme was increased when rabbits fed on copper oxychloride fungicide with pellets feeding. From the results in ALT enzyme of interaction between groups and periods showed that the lowest level in 1st group (control) was 17.05±1.20 u/l for 60 days, while the highest level of ALT enzyme was 84.99±1.20 u/l in 3rd group (treated with high level of copper) after 60 days from fed.

From the results presented in **Tables 4&5** and **Fig. 7**, it can be observed that, there were increased significantly differences ($P<0.001$) in ALT enzyme in rabbits, due to the effect of treatment. These results are in agreement with **Naoki *et al.* (1995) and Steve *et al.* (2000)** who found that, the rise of ALT enzyme in serum rats when exposed of copper dosed.

4-3-3-Creatinine:

The means of concentrations creatinine and urea in the serum reflect the changes in the kidney copper turnover and it apparently derived from this source.

Determination of creatinine and urea for assessment of kidney functions. The average level of creatinine in NZW rabbits under treatment is presented in **Table 4.**

The obtained results were 0.15±0.01, 0.36±0.01 and 0.55±0.01 mg/dl for 1st group (control), 2nd group and 3rd group for 60 days, respectively. The means of level creatinine in different periods for 20 days, 40 days and 60 days were 0.31±0.01, 0.34±0.01 and 0.40±0.01 mg/dl, respectively.

From the results presented in **Tables 4&5** and **Fig. 8**, it can be observed that, there were increased significantly differences ($P<0.001$) in creatinine level, due to the effect of treated with copper oxychloride fungicide. The results of interactions between groups and different periods, the lowest mean of creatinine was 0.14±.01 mg/dl in control group for 40 days, while the highest mean of creatinine level was 0.63±0.01 mg/dl in 3rd group (treated with high level of copper oxychloride fungicide) for 60 days. These results were increased significantly ($P<0.001$).

These results are in agreement with **Ayyat *et al.* (1991)** who found that in a study when treated rabbits with drinking salinity water (high level of heavy metals) caused a significant increase in serum creatinine level. And also with **Fisher (1992)** who found that in rabbits diet on copper poisoning lead to increase in serum creatinine level.

4-3-4-Urea:

The least square means of level serum urea in NZW rabbits showed in **Table 4** were 31.33±1.53, 29.75±1.53 and 28.60±1.53 mg/dl for 1st group (control), 2nd group (treated with low dose of copper as 0.1184 gm/kg.BW/day) and 3rd group (treated with high level of copper as 0.2368 gm/kg.BW/day) for 60 days, respectively. The mean levels of urea in different periods for 20, 40 and 60 days were 30.36±1.34, 26.39±1.49 and 32.40±1.72 mg/dl, respectively. The highest mean of urea level for interactions between groups and different periods was 33.88±2.99 mg/dl in the control group for 60 days and 23.39±2.59 mg/dl in 3rd group for 40 days.

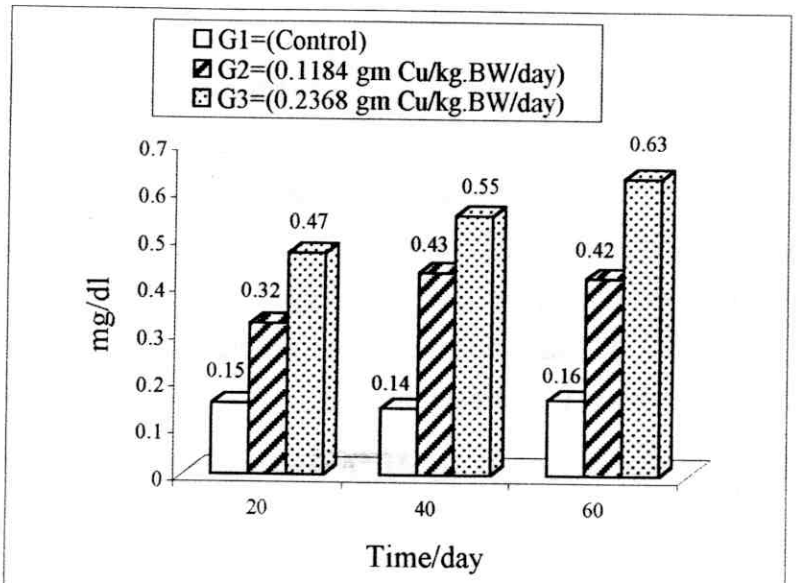

Fig 8: Effect of copper oxychloride fungicide on Creatinine

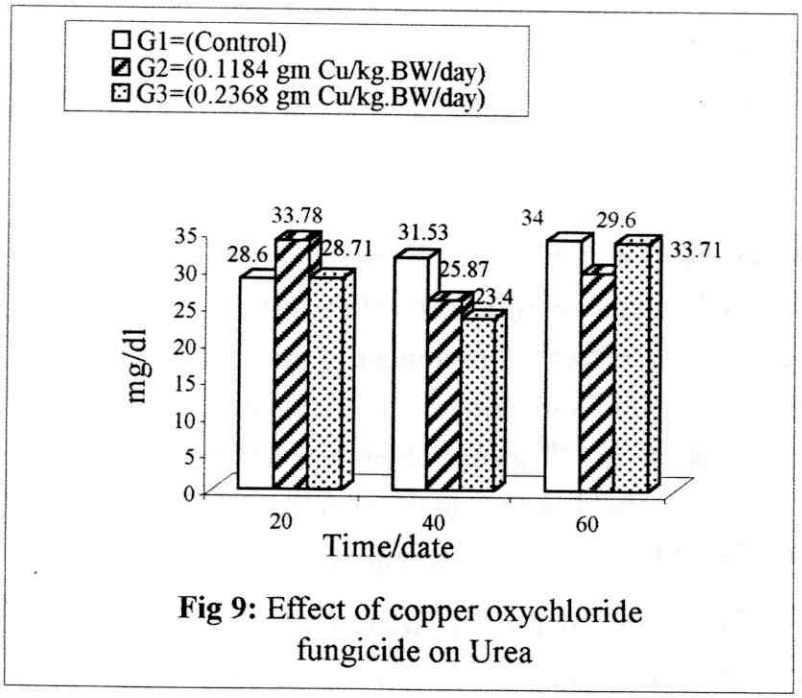

Fig 9: Effect of copper oxychloride fungicide on Urea

From the results presented in **Tables 4&5** and **Fig. 9**, it can be observed that, there were non-significant differences. These results are in disagreement with **Ahmed *et al.* (1998)** who reported that increased values of urea when treated rat animals with copper fungicide and with **Ayyat *et al.* (1991) and Fisher (1992)** who found that, level of serum urea was increased when treated of rabbits with heavy metals.

4-4-Copper residues in different tissues:

4-4-1-Serum:

The least square means of copper (Cu) residues in serum of the NZW rabbits under different treatment are presented in **Table 6** were 166.72±1.78, 222.10±1.78 and 376.69±1.78 ppm in the 1st group (control, feed without copper metal), 2nd group (treated with 0.1184 gm Cu/kg.BW/day) and 3rd group (treated with 0.2368 gm Cu/kg.BW/day) for 60 days, respectively.

The obtained results for different periods after 20 days, 40 days and 60 days were 198.36±1.56, 251.01±1.74 and 316.14±2.01 ppm, respectively. From the results presented in **Tables 6&7** and **Fig. 10**, it can be observed that, there were increased highly significantly ($P<0.001$) in cu concentrations, due to the effect of treatment. From the interaction results between groups and periods, the obtained highest mean of cu concentration in 3rd group which was 530.04±3.48 ppm after 60 days, while the lowest mean of cu concentration in serum was 146.27±2.70 ppm in the 1st group after 20 days.

The obtained results showed that Cu concentration was increased when rabbits fed on copper oxychloride fungicide as

Table 6: Least square means and standard error for copper intoxication on copper concentration in serum, liver, kidney, muscle, feces and urine in NZW rabbit does.

Items	Copper in Serum (µg/dl)	Copper in liver (ppm)	Copper in kidney (ppm)	Copper in muscle (ppm)	Copper in feces (ppm)	Copper in urine (ppm)
Main effects:						
Groups (G)						
1st Group	166.72±1.78^{c}	12.85±0.56^{c}	8.35±0.30^{c}	5.79±0.12^{c}	90.04+1.58^{c}	0.19±0.02^{c}
2nd Group	222.10±1.78^{b}	26.03±0.56^{b}	15.81±0.30^{b}	7.56±0.12^{b}	141.21+1.58^{b}	0.53±0.02^{b}
3rd Group	376.69±1.78^{a}	73.70±0.56^{a}	50.53±0.30^{a}	9.80±0.12^{a}	271.10+1.58^{a}	1.17±0.02^{a}
Periods (P):						
20 Days	198.36±1.56^{c}	26.00±0.56^{b}	24.60±0.30^{b}	7.40±0.12^{b}	137.72+1.38^{c}	0.48±0.01^{c}
40 Days	251.01±1.74^{b}	43.81±0.56^{a}	24.19±0.30^{b}	7.80±0.12^{a}	174.82+1.54^{b}	0.76±0.01^{a}
60 Days	316.14±2.01^{a}	42.76±0.56^{a}	25.89±0.30^{a}	7.94±0.12^{a}	189.81+1.78^{a}	0.64±0.02^{b}
Interaction:						
G_1 x 20	146.26±2.7^{g}	7.10 ±0.98^{f}	7.83±0.52^{d}	5.79±0.20^{e}	67.31±2.39^{g}	0.25±0.02^{g}
G_1 x 40	167.79±3.02^{f}	16.73±0.98^{ed}	8.45±0.52^{d}	5.83±0.20^{e}	99.38±2.67^{f}	0.22±0.03^{h}
G_1 x 60	186.11±3.48^{e}	14.72±0.98^{e}	8.76±0.52^{d}	5.73±0.20^{e}	103.42±3.08^{f}	0.17±0.03^{h}
G_2 x 20	219.32±2.7^{d}	18.10±0.98^{d}	16.80±0.52^{c}	7.04±0.20^{d}	133.58±2.39^{d}	0.33±0.02^{f}
G_2 x 40	214.73±3.01^{d}	29.41±0.98^{c}	15.38±0.52^{c}	7.69±0.20^{c}	139.40±2.67^{e}	0.58±0.03^{e}
G_2 x 60	232.26±3.48^{c}	30.58±0.98^{c}	15.27±0.52^{c}	7.96±0.20^{c}	150.64±3.08^{e}	0.66±0.03^{d}
G_3 x 20	229.51±2.7^{c}	52.80±0.98^{b}	49.18±0.52^{b}	9.38±0.20^{b}	195.21±2.39^{c}	0.85±0.02^{c}
G_3 x 40	370.52±3.02^{b}	82.31±0.98a	48.76±0.52^{b}	9.89±0.20^{ab}	285.68±2.67^{b}	1.10±0.03^{a}
G_3 x 60	530.04±3.48^{a}	85.99±0.98a	53.66±0.52^{a}	10.13±0.20^{a}	332.42±3.08^{a}	1.55±0.03^{b}

Means with the same letter within each column in each factor are non-significantly different ($P < 0.05$).
1st group (G_1)= Control, 2nd group (G_2) = (0.1184 gm Cu/kg. BW/day) and 3rd group (G_3) = (0.2368 gm/kg. BW/day.

Table 7: F-ratio of analysis of variance for the effect of copper concentration in serum, liver, kidney, muscle, feces and urine in NZW doe rabbits.

S.O.V	df.	copper in serum	copper in liver	copper in kidney	copper in muscle	copper in feces	copper in urine
Group (G)	2	3736.17***	3230.11***	5631.59***	303.37***	3509.46***	1140.89***
Period (P)	2	1079.16***	314.93***	8.75**	5.84*	310.78***	109.49***
GXP	4	662.73***	53.86***	11.05***	1.72	224.36***	83.03***
Error df	63						
Error MS		72.83	1.90	0.54	0.08	57.05	0.01

* = Significant ($P < 0.05$).
** = Significant ($P < 0.01$).
*** = Highly significant ($P < 0.001$).

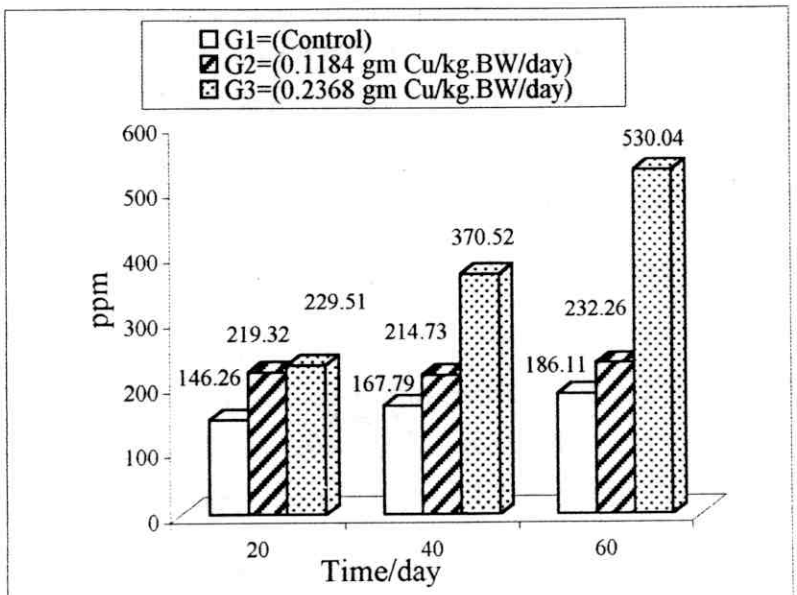

Fig 10:Copper concentration in serum

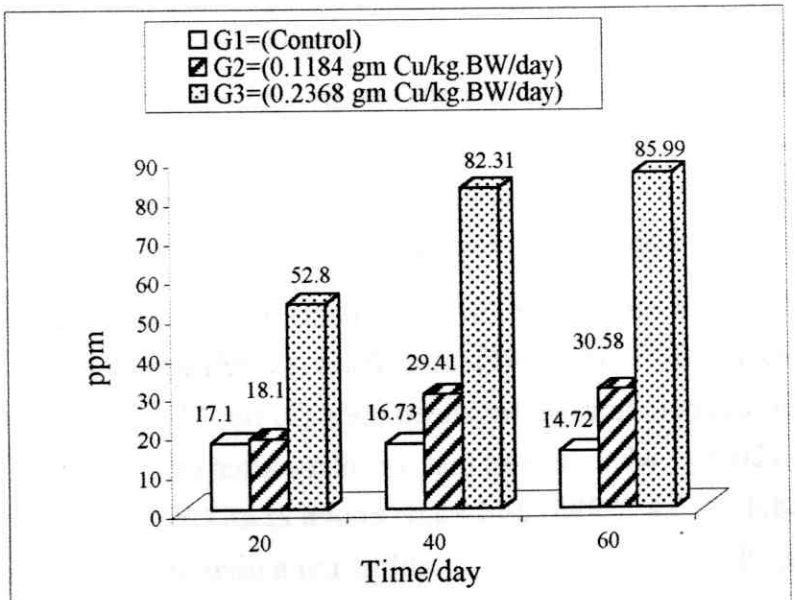

Fig 11: Copper concentration in liver

(0.2368 gm Cu/kg. BW/day). These results are in agreement with **Lal & Sourkes (1971) and Underwood (1977)** who reported that, the copper concentration increased in serum sheep by fed on copper with advanced time.

4-4-2-Liver:

The obtained results in **Table 6** showed that the mean values of copper concentration in the examined liver rabbit samples were 12.85±0.56, 26.03±0.56 and 73.70±0.56 ppm for 1st group, 2nd group and 3rd group for 60 days, respectively.

The least square means values of Cu concentration in the liver rabbits in different periods were 26.00±0.56, 43.81±0.56 and 42.76±0.56 ppm for 20 days, 40 days and 60 days, respectively. From the results presented in **Tables 6&7** and **Fig.11**, it can be observed that, there were increased Cu concentration in liver rabbits and highly significantly ($P<0.001$), due to the effect of fed on Cu oxychloride. From the results of interaction for mean Cu residue in the liver rabbits, the highest mean was showed in the 3rd group which was 85.99±0.98 ppm for 60 days, while the obtained lowest mean of Cu residue was 17.10±0.98 ppm in 1st group (control).

These results are in agreement with **Underwood (1977); Buckley & Tait (1981) and Diab (1995)** who found that, the liver copper storage in sheep diets Cu for 77 days; **Miranda *et al.* (2000)** who showed that, in calves after feeding on Cu diets; **Abdel-Hafez (1995) and Skrivanova *et al.* (2002)** who reported that, the high Cu level in rabbits fed a diets supplemented with copper at 140 mg cu/kg feed.

4-4-3-Kidney:

The least square means of copper residues (Cu) in the examined kidney samples under different treatment are presented in **Table 6** were 8.35±0.30, 15.81±0.30 and 50.53±0.30 ppm for 1st group, 2nd group and 3rd group for 60 days, respectively. These results presented in **Tables 6&7** and **Fig. 12**, it can be observed that, there were increased highly significantly ($P<0.001$) in Cu residues in kidney, due to the effect of treatment.

The least square means of Cu residues in kidney rabbits at different periods for treatment were 24.60±0.30, 24.19±0.30 and 25.89±0.30 ppm for 20 days, 40 days and 60 days, respectively. From the results obtained in different periods, there were significant differences ($P<0.01$) in cu concentration in kidney for treated groups compared with control group.

The least square means of Cu residue in kidney rabbits for interaction between groups and different periods in **Table 6**, the highest value was in 3rd group (fed on 0.2368 gm Cu / kg. BW/day) for 60 days, while the lowest value which was 7.83±0.52 ppm in the control group for 20 days. The obtained results showed that Cu concentration in kidney tissues was increased when rabbits fed on Cu diets (0.2368 gm Cu/kg.BW/day) for 60 days. These results are in agreement with **Hore *et al.* (1997)** who found that, the kidney rat animals storage for Cu when exposured fungicide for 49 days and with **Adel-Hafez (1995)** who observed that, Cu residues in NZW rabbit kidneys when given feed copper.

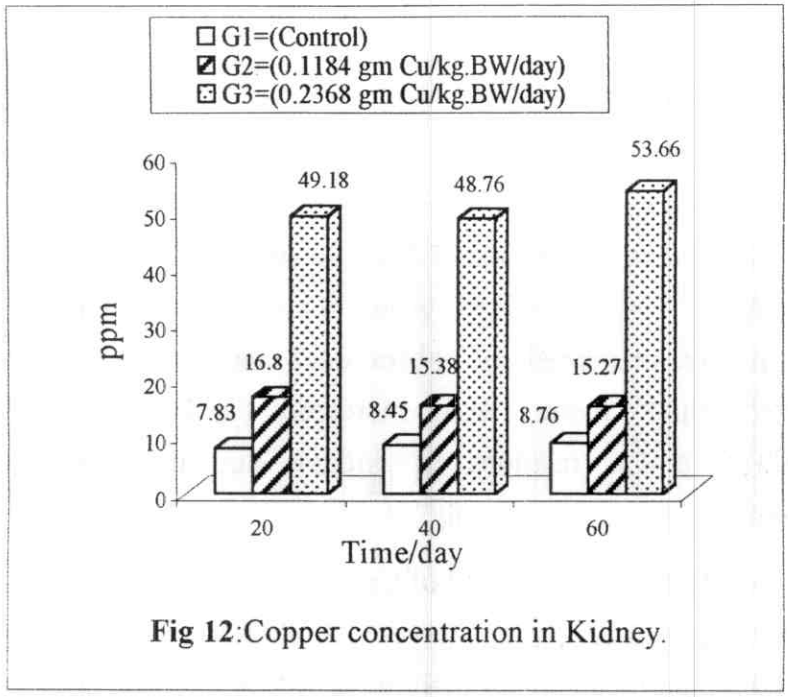

Fig 12:Copper concentration in Kidney.

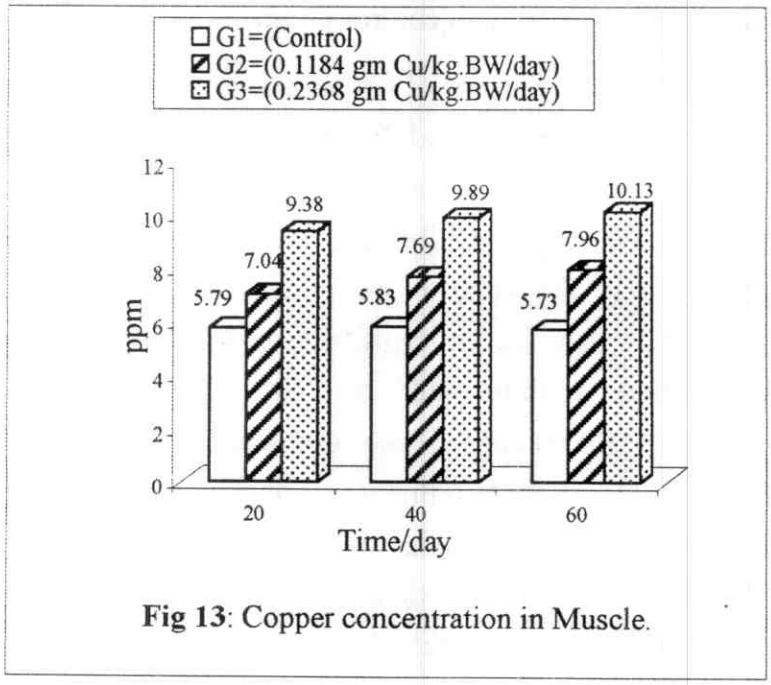

Fig 13: Copper concentration in Muscle.

4-4-4-Muscle:

The mean values of copper (Cu) residue in muscle of NZW rabbits under different treatment are presented in **Table 6**, the results obtained were 5.79±0.12, 7.56±0.12 and 9.80±0.12 ppm for 1st group (control), 2nd group (fed on 0.1184 gm Cu/kg.BW/day) and 3rd group (fed on 0.2368 gm Cu/kg.BW/day) for 60 days, respectively. From the results presented in **Tables 6&7** and **Fig. 13**, it can be observed that, there were increased significantly ($P<0.001$) in Cu concentration in muscle tissues for NZW rabbits, due to the effect of treatment.

The results obtained in different periods 20 days, 40 days and 60 days were 7.40±0.12, 7.80±0.12 and 7.94±0.12 ppm Cu in muscle tissues, respectively, these results were significant increased at ($P<0.05$). From the results for interaction between groups and periods, it was found that the highest of mean Cu residue in muscle tissues was 10.13±0.20 ppm in the 3rd group for 60 days, while the lowest of mean Cu residue in was 5.73±0.20 ppm in control group rabbits for 60 days.

These results are in agreement with **Abdel-Hafez (1995) and Skrivanova *et al.* (2002)** who reported that, the total mean copper in examined muscle samples in rabbits during a chronic exposure to diets with different Cu levels were increased significantly in the Cu concentration in rabbit muscle tissues

4-5-Copper excretion:

4-5-1-Feces:

The obtained results presented in **Table 6** showed, the mean values of copper (Cu) concentration in feces for NZW

rabbits under different treatment were 90.04±1.58, 141.21±1.58 and 271.10±1.58 ppm for 1st group (control), 2nd group (fed on 0.1184 gm Cu/kg.BW/day) and 3rd group (fed on 0.2368 gm Cu/kg.BW/day) for 60 days, respectively and the obtained results in different periods at 20 days, 40 days and 60 days, the means of Cu concentration in feces were 137.72±1.38, 174.82±1.54 and 189.81±1.78 ppm Cu in feces rabbits, respectively.

From the results for interaction between groups and periods, the observed of high mean value Cu concentration in feces which was 332.42±3.08 ppm in the 3rd group for 60 days, while the lowest mean value for Cu concentration in feces was 67.31±2.39 ppm in the control group for 20 days. The results presented in **Tables 6&7** and **Fig. 14**, it can be observed that, there were increased significantly differences ($P<0.001$) in Cu concentration in feces, due to the effect of treatment.

These results are in agreement with **Bauer (1982)** who reported that, the copper levels in feces from rabbits fed copper sulfate was elevated and suggested that much of the dietary copper eliminated from body by this route; **Abdel-Hafez (1995) and Skrivanova *et al.* (2002)** who found that very high copper concentration in feces when rabbits fed on coppered diets.

4-5-2-Urine:

The mean concentrations of urinary copper in NZW rabbits under different treatment as shown in **Table 6**, were 0.19±0.02, 0.53±0.02 and 1.17±0.02 ppm in 1st group, 2nd group and 3rd group for 60 days respectively. The mean values obtained in different periods for 20 days, 40 days and 60 days were 0.48±0.01, 0.76±0.01 and 0.64±0.02 ppm cu in urine,

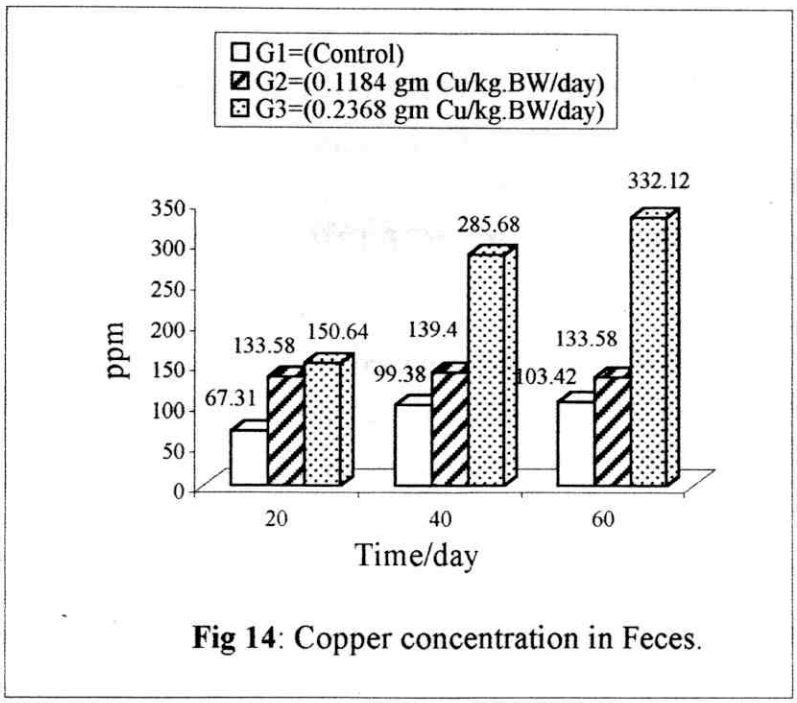

Fig 14: Copper concentration in Feces.

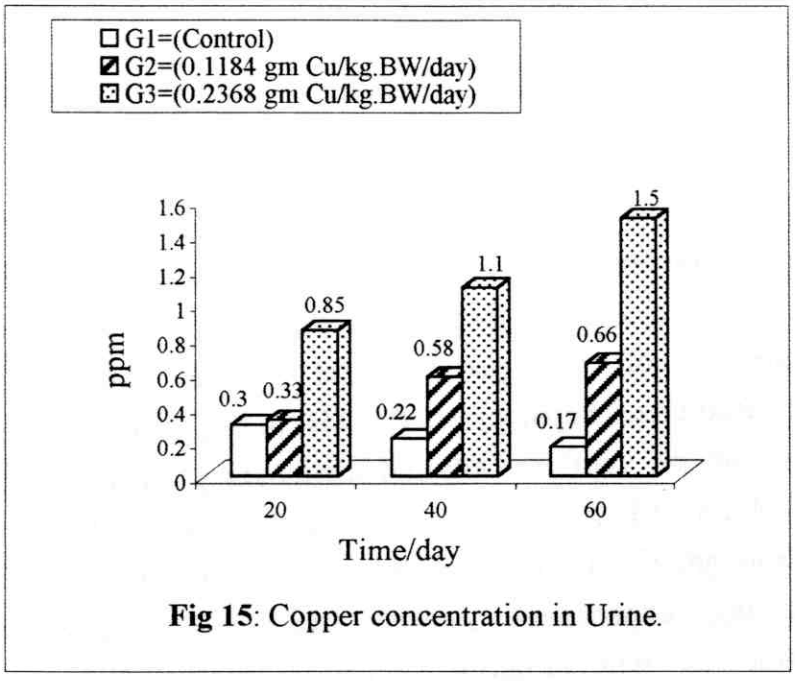

Fig 15: Copper concentration in Urine.

respectively. And the results observed in **Table 6**, showed the highest mean value of Cu concentration in urine was 1.55±0.03 ppm in 3rd group for 60 days, while the lowest mean value of Cu concentration in urine was 0.17±0.03 ppm Cu in control group for 60 days.

From the results presented in **Tables 6&7** and **Fig. 15**, it can be observed that, there were increased significantly ($P<0.001$) in Cu concentration in urine, due to the effect of the treatment.

The obtained results showed that, Cu concentration in urine was increased when rabbits fed on 0.2368 gm Cu/kg.BW/day for 60 days. These results agreed with **Bauer (1982) and Abdel-Hafez (1995)** who found that in rabbits fed copper sulfate and copper oxychloride, the results were increased significant in urinary copper level between groups.

4-6-Histopathological changes:

4-6-1-Liver:

The microscopic examination of the liver of rabbits fed on copper oxychloride (0.1184 gm Cu/kg. BW/day) as 1/20 LD_{50} for 60 days from beginning experiment revealed slight congestion of the portal blood vessels with hydropic degeneration of hepatocytes (H.D.H) in **Fig. 16&17** Moreover, activation of kupffer's cells and slight hyperplasia of bile ductal epithelium (B.D.E) were seen. Also, in the 3rd goupe treated with (0.2368 gm Cu/BW/day) in fed as 1/10 LD_{50} for 60 days from beginning experiment showed hydropic degeneration of hepatocytes with inflatory cellular infiltration of the hepatic

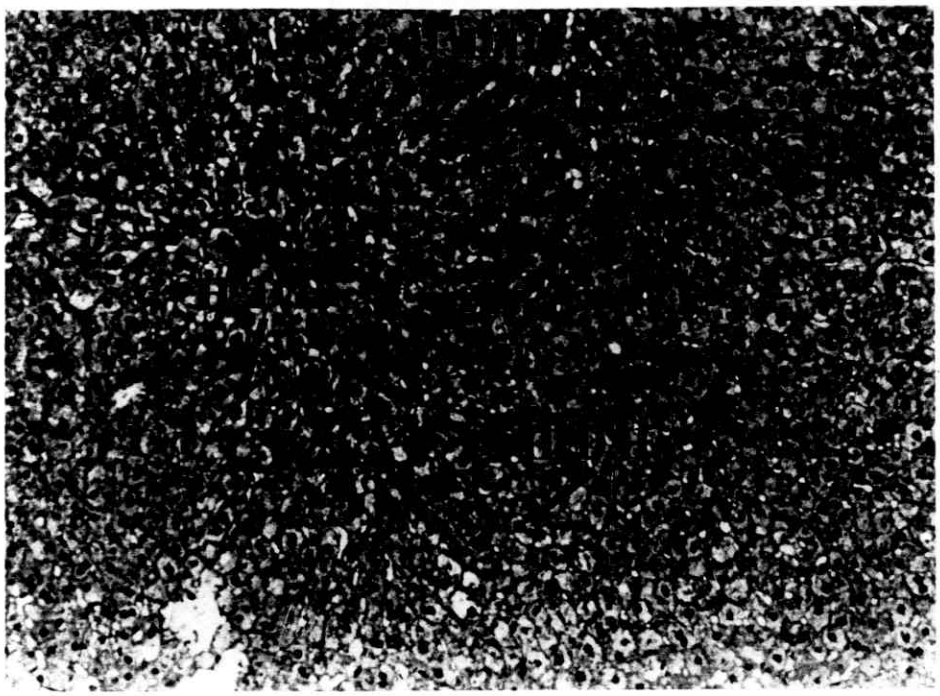

Fig.16 : Cross section in liver of NZW rabbits fed on copper oxychloride 0.1184 gm Cu/kg BW/day) as 1/20 LD_{50} for 40 days.Arrows indicate hydropic degeneration of hepatocytes (H.D.H). (H&E X200).

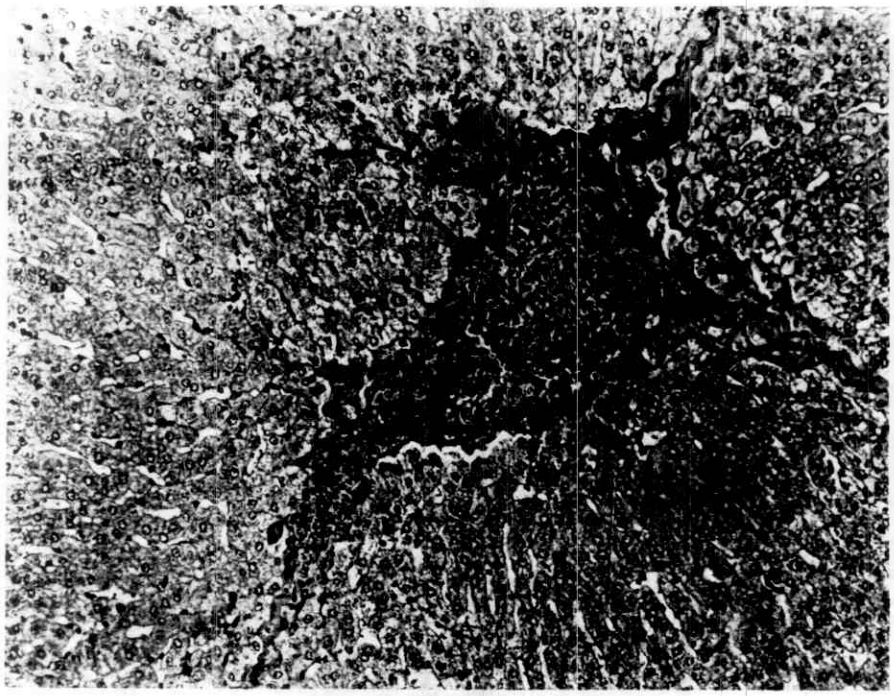

Fig.17: Cross section in liver of NZW rabbits fed on copper oxychloride (0.1184 gm Cu/kg.BW/day) as 1/20 LD_{50} for 60 days. Arrow indicates focal aggregation (F.A.L.) of Lymphocytes (H&E X 200).

parenchyma mainly lymphocytes were showed in **Fig. 18&19** Sever hyperplasia of the lining epithelium of the bile ducts with periductal fibrosis, mononuclear inflammatory cellular aggregation and formation of newly bile ductless. The results for histopathological changes due to copper toxicity in the liver agreement with observed by **Pimental & Menezes (1975) and Fisher (1992)** who reported that the development extensive liver damage with fibrosis, micronodular crrhiosis, angiosar coma and portal hypertention, have been seen with chronic exposure to copper sulfate in sheep; **Haywood (1979) and Hore *et al.* (1997)** recorded that, the signs of copper toxicity due to the most highly supplement diets in rats were early liver damage and pathological changes significantly, and **Abdel-Hafez (1995)** who showed in NZW rabbits fed highly copper diets, the pathological in showed significant changes in the liver.

4-6-2-Kidney:

The histopathological changes due to copper toxicity in the kidney of rabbits were multiple areas of cloudy swelling of the lining epithelium (C.S.L.E) of some renal convoluted tubules were only the microscopical changes observed in the examined kidney of rabbits fed on (0.1184 gm Cu/kg. BW/day) copper oxychloride as 1/20 LD_{50} for 60 days from beginning experiment in **Fig. 20** of the 2nd group. But in the 3rd group fed on (0.236 gm Cu/kg. BW/day) copper oxychloride as 1/10 LD_{50} for 60 days showed that characterized by congestion of the renal blood vessels and intratubular capillaries with degeneration of the lining epithelium of renal tubules in the form of cloudy swelling and vacublar degeneration **Fig. 21**. These results agreed

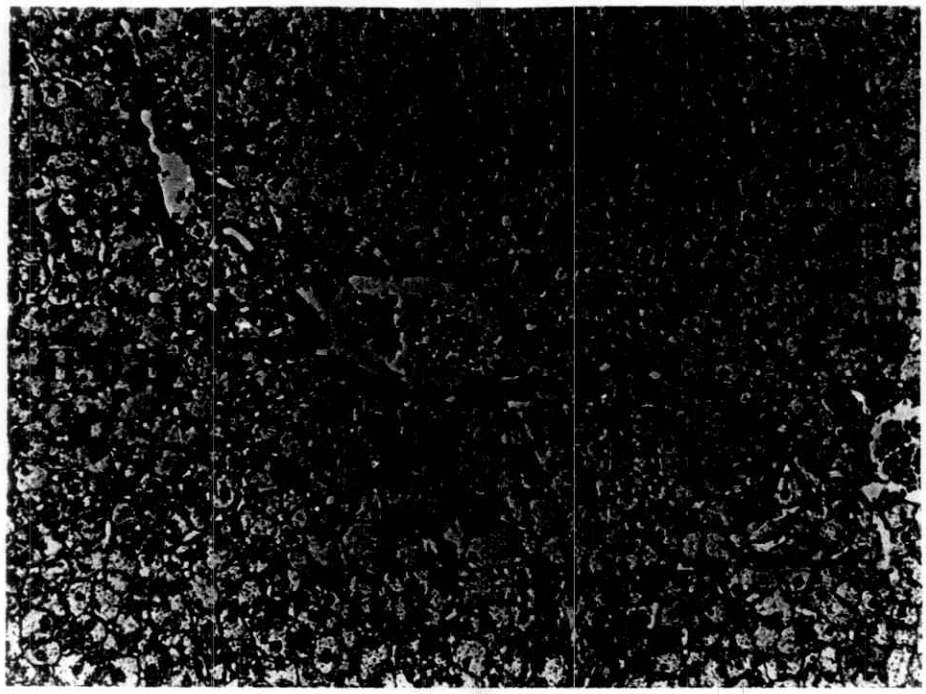

Fig.18: Cross section in liver of NZW rabbits fed on copper oxychloride (0.236 gm Cu/kg. BW/day) as 1/10 LD_{50} for 40 days. Arrows indicate extesive hydropic degeneration of hepatocytes (H.D.H) and congestion of central veins (C.V). (H&E X200).

Fig.19: Cross section in liver of NZW rabbits fed on copper oxychloride (0.2368 gm Cu/kg. BW/day) as 1/10 LD_{50} for 60 days. Arrows indicate sever hyperplasia of lining epithelium (L.E) of the bile ducts (B.D) with periductal fibrosis. (H&EX200).

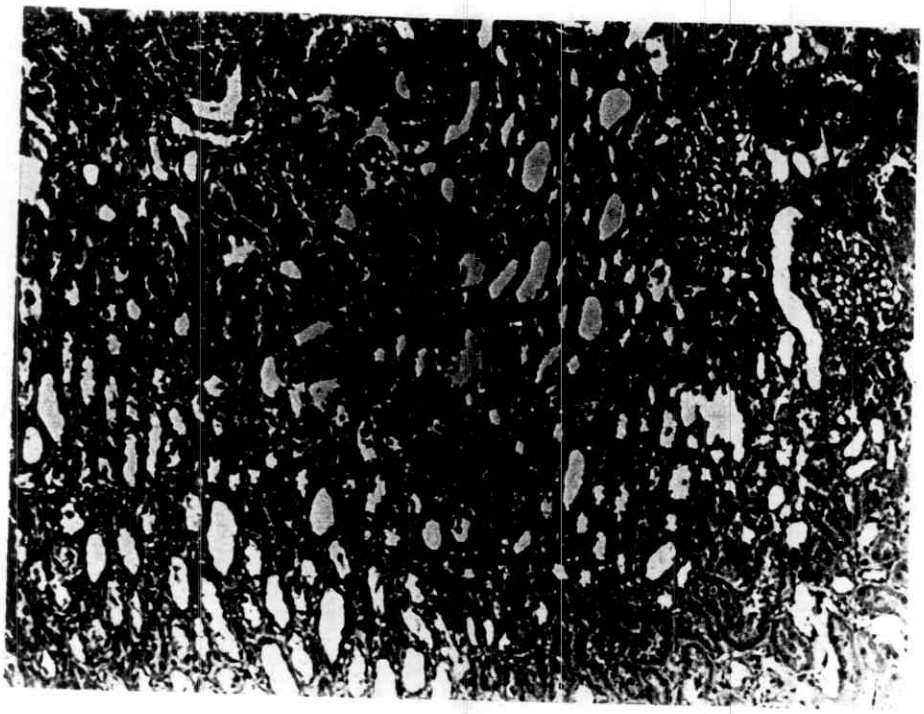

Fig. 20: Cross section in kidney of NZW rabbits fed on copper oxychloride 0.1184 gm Cu/kg. BW/day) as 1/20 LD_{50} for 60 days. Arrows indicate cloudy swelling of the lining epithelium of renal convoluted (C.S.L.E) tubules. (H&E X200)

Fig. 21: Cross section in kidney NZW rabbits fed on copper oxychloride (0.236 gm Cu/kg. BW/day) as 1/10 LD_{50} for 60 days. Arrows indicate cloudy swelling (C.S) and vacuolar degeneration of the renal tubules (V.D.R.T). (H&EX200).

with those observed by **Adams *et al.* (1977) and Wilhelmsen (1979)** who showed that in chronic copper poisoning of goats and sheep histological changes in kidneys and showed many tubular epithelial cells were under going degeneration; **Haywood (1985) and Hore *et al.* (1997)** who showed that, the pathological significant changes in the kidney for rats. **Abdel-Hafez (1995)** who observed in NZW rabbits the kidneys were soft and pathological changes in the kidney tissues.

4-6-3-Ovary:

The trace elements of the fungicide produced considerable changes of the ovarian follicular structures and the corpora lutea. The interrelations of morphological and functional changes with increased occurrences of trace elements in various tissues necessitate further investigation.

The microscopical examination of the ovaries in the 2nd group rabbits fed on (0.1184 gm Cu/kg. BW/day) as 1/20 LD_{50} copper oxychloride for 60 days revealed normal histological picture the ovary with presence of the normal immature ovarian follicles **(Fig. 22),** while in the 3rd group, when rabbits fed on (0.2368 gm Cu/kg. BW/day) as 1/10 LD_{50} copper oxychloride for 60 days from beginning experiment, the histopathological examination of ovaries revealed nearly normal of histological ovarian tissues. However degeneration and atrasia of some ovarian follicles were seen **(Fig. 23)**. These results agreed with the finding of **Bires *et al.* (1995)** who showed in sheep that, diet of copper intoxication, the histological changes in the number of ovarian follicles and increased occurrence of primary atretic follicles; **Ahmed *et al.* (1998)** showed that, copper treated rats

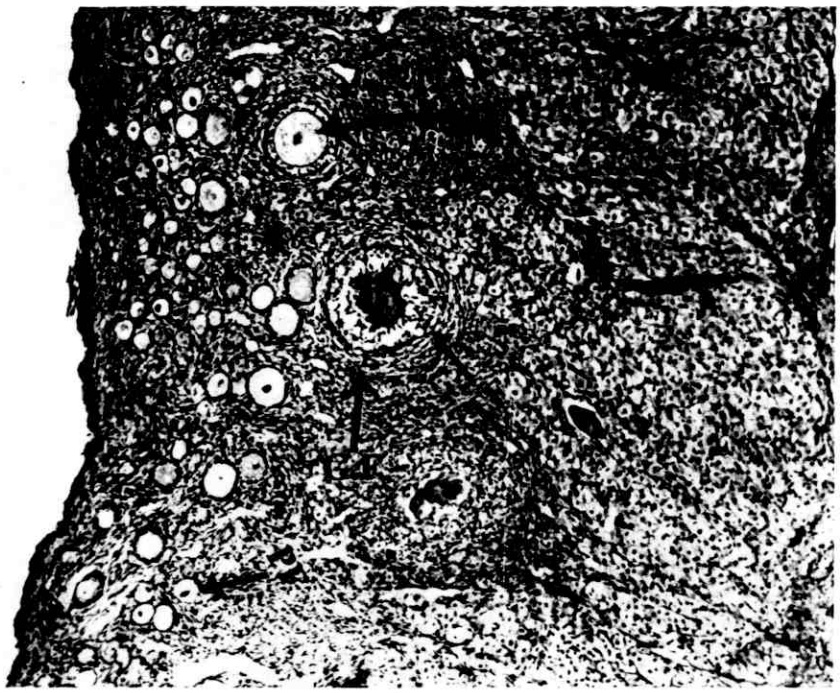

Fig. 22: Cross section in ovary of NZW rabbits control group. Arrows indicate normal of primary follicles (PF), secondary follicles (SF) , graffian follicles (GF) and theca folliculi (TF) in cortex and blood vessels (BV) in stroma (ST). (H&EX200).

Fig. 23: Cross section in ovary NZW rabbits fed on copper oxychloride (0.2368 gm Cu/kg. BW/day) as 1/10 LD_{50} for 60 days. Arrows indicate degeneration and atrasia of some ovarian follicles (D.A.O.F.),(H&EX 200).

revealed absence of mature graafian follicles and decreased number of growing follicles. **Niswender et al. (1985); Wolfenson & Orlyblum (1988) and Kliment & Zithy (1989)** who showed that in rabbits treated with copper, small and large which originate from granulosa and theca cells.

4-6-4-Uterine horn:

Congestion of the uterine blood vessels, focal areas of endometrial hemorrhages and hyperplasia of the endometrial lining epithelium were seen in the examine uterine horn of rabbits in 2nd group fed on (0.1184 gm Cu/kg. BW/day) copper oxychloride for 60 days **Fig. 24**. In the 3rd group rabbits fed on (0.2368 gm Cu/kg. BW/day) copper oxychloride for 60 days from start of the experiment, the microscopic examination of the uterine horn revealed non-suppurative endometritis characterized by congestion of uterine blood vessels and capillaries **Fig. 25**, with hyperplasia of the epithelium lining endometrium and leucocytic cellular infiltration of the uterine wall mostly lymphocytes.

These results agreed with the finding of **Bires *et al.* (1995)** who showed that in ewes fed on high level copper diets, the copper (Cu) concentration in uteri and changes pathological endometrium. **Ahmed *et al.* (1998)** who showed that microscopical examination of uterus of copper treated female rats, the surface epithelial cells showed focal hyperplasia, uterine glands showed degenerative and necrotizing changes of their epithelium lining, that results due to exposured copper oxychloride fungicide.

Fig. 24: Cross section in uterine horn for NZW rabbits fed on copper oxychloride (0.1184 gm Cu/kg.BW/day) as 1/20 LD_{50} for 60 days. Arrows indicate congestion of the uterine blood vessels. (H&E X 200).

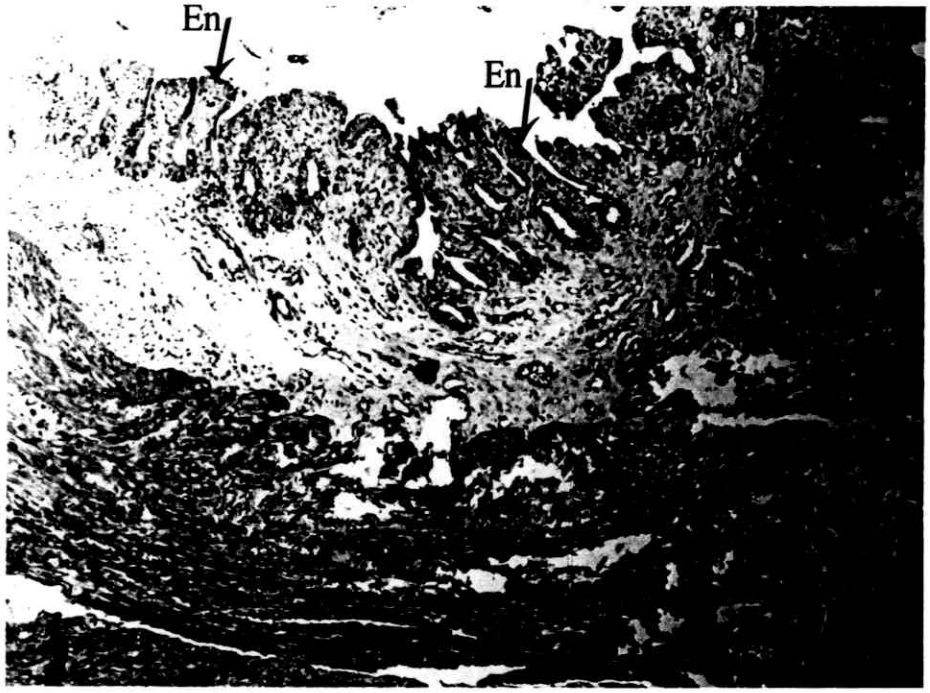

Fig. 25: Cross section in uterine horn for NZW rabbits fed on copper oxychloride (0.2368 gm Cu/kg.BW/day) as 1/10 LD_{50} for 60 days. Arrows indicate hyperplasia of the lining epithelium of endometrium (En),(H&E X 200).

4-7-Reproductive and productive performance:

4-7-1-Ovarian weight:

Means of ovaries weight (left and right) of pregnant NZW female rabbits under different treatment with exposure to copper oxychloride fungicide in feed for different periods, are presented in **Table 8**. Means of left ovaries were 0.24, 0.24 and 0.23 gm in the 1st , 2nd and 3rd group, respectively, however, the means of right ovaries weights were 0.26, 0.26 and 0.24 gm for the same groups, respectively. **Table 9**, showed that, there were no significant differences in ovaries weight, due to treatment.

The means of left ovaries weights were 0.24, 0.24 and 0.23 gm while these of right ovaries weights were 0.25, 0.27 and 0.24 gm after 20, 40 and 60 days of feeding respectively. The highest mean value in the left ovaries weight was 0.26 gm in the 1st group (control) for interaction between groups and periods, while the lowest mean value was 0.22 gm in the 3rd group after 20 days. And the highest mean value in right ovaries was 0.28 gm in the 1st group after 40 days, while the lowest mean value was 0.24 gm in the same group after 60 days. The observed results for effect of copper oxychloride fungicide on ovarian weight were non-significant in **Tables 8&9**.

These results are in agreement with **Hafez and Rajakoski (1964)** who found that, the average weight of each ovarian pair in multiparous NZW rabbits at day 6 after mating ranged from 266 to 892 mg with average of 479 mg. **Jeanson *et al.* (1981)** who observed that, the weights of right and left ovaries in NZW rabbits at 6 days post-coitum ranged 178-489 mg, and **El-Terbany (1993)** who found that, the average of ovarian weight

Table 8: Least square means and standard error for copper intoxication on ovary weight and oviduct length in NZW doe rabbits.

Items	Ovary weight		Oviduct length	
	left (gm)	right (gm)	left (cm)	right (cm)
Main effects:				
Groups (G):				
1st Group (G_1)	0.24±0.01[a]	0.24±0.01[a]	10.29±0.83[a]	10.87±0.20[a]
2nd Group (G_2)	0.24±0.01[a]	0.24±0.01[a]	11.04±0.83[a]	11.35±0.20[a]
3rd Group (G_3)	0.23±0.01[a]	0.23±0.01[a]	10.57±0.83[a]	10.87±0.20[a]
Periods (P):				
20 Days	0.24±0.01[a]	0.24±0.01[a]	10.69±0.83[a]	11.15±0.20[a]
40 Days	0.24±0.01[a]	0.24±0.01[a]	9.19±0.83[a]	10.99±0.20[a]
60 Days	0.23±0.01[a]	0.23±0.01[a]	10.74±0.83[a]	10.95±0.20[a]
Interaction				
G_1 x 20	0.26±0.02[a]	0.26±0.02[a]	10.96±1.44[a]	10.76±0.34[a]
G_1 x 40	0.24±0.02[a]	0.24±0.02[a]	9.12±1.44[a]	10.85±0.34[a]
G_1 x 60	0.22±0.02[a]	0.22±0.02[a]	10.80±1.44[a]	11.00±0.34[a]
G_2 x 20	0.25±0.02[ab]	0.25±0.02[ab]	11.05±1.44[a]	11.69±0.34[a]
G_2 x 40	0.26±0.02[ab]	0.26±0.02[ab]	11.31±1.44[a]	11.25±0.34[a]
G_2 x 60	0.23±0.02[ab]	0.23±0.02[ab]	10.75±1.44[a]	11.10±0.34[a]
G_3 x 20	0.22±0.02[a]	0.22±0.02[a]	10.05±1.44[a]	11.00±0.34[a]
G_3 x 40	0.22±0.02[ab]	0.22±0.02[ab]	11.01±1.44[a]	10.86±0.34[a]
G_3 x 60	0.24±0.02[a]	0.24±0.02[a]	10.67±1.44[a]	10.75±0.34[a]

Mean with the same letter within each column in each factor are non-significantly different ($P < 0.05$).

G_1 =Control ; G_2=fed on 0.1184 gm Cu/kg. BW/day;G_3=fed on 0.2368 gm Cu/kg. BW/day.

Table 9: F-ratio of analysis of variance for the effect of copper intoxication on ovary weight and oviduct length in NZW doe rabbits.

S.O.V	df.	Ovary weight		Oviduct length	
		left (gm)	right (gm)	left (cm)	right (cm)
Group (G)	2	0.84	0.91	1.64	1.92
Period (P)	2	0.24	0.97	1.12	0.28
G X P	4	1.03	0.46	2.07	0.38
Error (P)	9				
Error MS		0.001	0.001	4.12	0.24

for rabbits born during summer season was0.46 gm at the age of sexual maturity.

4-7-2-Oviducts length:

The mean values of left and right oviducts length of NZW rabbits under different treatment in **Table 8** showed that, the values of left oviducts length were 10.29, 11.04 and 10.57 cm for 1st, 2nd and 3rd group respectively, while the means of right oviducts length were 10.87, 11.35 and 10.87 cm for the same groups. With respect of the effect for periods, it was found that the oviduct length after 20, 40 and 60 days from treatment periods were 10.69, 9.19 and 10.74 cm respectively. While the mean values of right oviducts length were 11.15, 10.99 and 10.95 cm after 20, 40 and 60 days from treatment respectively.

From the results of interaction between groups and periods, the highest and the lowest mean values of left oviducts length were 11.31 cm in 2nd group and 9.12 cm in the 1st group after 40 days of treatment, respectively. The highest mean values of right oviducts length was 11.69 cm in 2nd group after 20 days, while the lowest mean value was 10.75 cm in the 3rd group after 60 days. From the results presented in **Tables 8&9**, it can be observed that, there were non-significant in oviduct length.

These results agreed with **Radwan and El-Sayaad (1990) and Abdel-Kafy (2000)** who reported that, the longest length of oviduct was obtained for Giza White rabbits, while the shortest was recorded for Californian (Cal) and rabbits, and oviducts weight in NZW rabbits (age 6 months) was 227.5 mg at 48 hours post-coitum.

4-7-3-Corpus luteum:

The mean numbers of corpora lutea in the left ovaries were 3.67, 3.33 and 4.00 for 1st, 2nd and 3rd group respectively. These results were significantly differences in number of corpora lutea in left ovaries, while the mean numbers of corpora lutea in the right ovaries were 5.00, 5.00 and 5.67 in the same groups respectively in **Table 10**. These results were non-significant between groups.

The obtained results for mean numbers of corpora lutea in the left ovaries for different periods after 20, 40 and 60 days were 3.17, 3.00 and 2.33 respectively. While the mean values of number corpora lutea for right ovaries were 4.33, 3.83 and 3.83 in the same periods. These results were decreased significantly ($P<0.05$) for number of corpora lutea in right ovaries.

From the results presented for interaction between groups and periods, showed that the highest mean of number corpora lutea for left ovaries was 4.00 in the control group at 20 days, while the lowest value was 3.00 in the 2nd group for the same period. The highest mean number of corpora lutea in the right ovaries was 7.50 after 20 days and the lowest mean value was 4.00 in number of corpora lutea in the 3rd group after 40 days of treatment in **Table 10.** The interaction results were decreased significantly differences ($P<0.01$) in number of corpora lutea in the right ovaries in **Table 11**. These results agreed with the finding of **Hafez and Rajakoski (1964)** who found that, ovulation rate in NZW female rabbits ranged from 3 to 25 with an average of 10 ova. And also with **El-Fouly et al (1977)** who reported that, in Bouscat and Egyptian Giza White rabbits

Table 10: Least square means and standard error for copper intoxication on numbers of corpora lutea and embryos in NZW doe rabbits.

Items	Number of corpora lutea		Number of embryos	
	left ovary	right ovary	left horn	right horn
Main effects:				
Groups (G):				
1st Group (G_1)	3.67±0.19^{ab}	5.00±0.24^{a}	3.50±0.26^{a}	4.83±0.39^{a}
2nd Group (G_2)	3.33±0.19^{b}	5.00±0.24^{a}	2.50±0.26^{b}	3.67±0.39^{ab}
3rd Group (G_3)	4.00±0.19^{a}	5.67±0.24^{a}	2.50±0.26^{b}	3.50±0.39^{b}
Periods (P):				
20 Days	3.67±0.19^{a}	5.83±0.24^{a}	3.17±0.26^{a}	4.33±0.39^{ab}
40 Days	3.67±0.19^{a}	4.83±0.24^{b}	3.00±0.26^{a}	3.83±0.39^{a}
60 Days	3.67±0.19^{a}	5.00±0.24^{b}	2.33±0.26^{a}	3.83±0.39^{a}
Interaction				
G_1 x 20	4.00±0.33^{a}	4.50±0.41^{ab}	3.50±0.44^{a}	4.50±0.67^{ab}
G_1 x 40	3.50±0.33^{a}	5.50±0.41^{b}	3.50±0.44^{a}	5.00±0.67^{a}
G_1 x 60	3.50±0.33^{a}	5.00±0.41^{bc}	3.50±0.44^{a}	5.00±0.67^{a}
G_2 x 20	3.00±0.33^{a}	5.50±0.41^{b}	2.50±0.44^{a}	5.00±0.67^{a}
G_2 x 40	3.50±0.33^{a}	5.00±0.41^{bc}	3.00±0.44^{a}	3.50±0.67^{ab}
G_2 x 60	3.50±0.33^{a}	4.50±0.41^{bc}	2.00±0.44^{a}	2.50±0.67^{b}
G_3 x 20	4.00±0.33^{a}	7.50±0.41^{a}	3.50±0.44^{a}	3.50±0.67^{ab}
G_3 x 40	4.00±0.33^{a}	4.00±0.41^{c}	2.50±0.44^{ab}	3.00±0.67^{ab}
G_3 x 60	4.00±0.33^{a}	5.50±0.41^{b}	1.50±0.44^{b}	4.00±0.67^{ab}

Means with the same letter within each column in each factor are non-significantly different ($P<0.05$).
G_1 =Control; G_2 =fed on 0.1184 gm Cu/kg. BW/day; G_3 =fed on 0.2368 gm Cu/kg. BW/day.

Table 11: F-ratio of analysis of variance for copper intoxication on numbers of corpora lutea and embryos in NZW doe rabbits.

S.O.V	df.	Number of corpora lutea		Number of embryos	
		left ovary	right ovary	left horn	right horn
Group (G)	2	3*	2.67	5.14*	3.56*
Period (P)	2	0	5.17*	3	0.56
G X P	4	0.75	8.17**	1.71	1.88
Error (P)	9				
Error MS	0.001	0.22	0.33	0.39	0.89

* = Significant ($P < 0.05$).
** = Significant ($P < 0.01$).

number of counted corpus luteum was significantly lower at days 3 post-coitum (7.7) than determined at 12 days after mating (8.4).And **Hulot & Mariana (1985)** who showed that, 8 hours after mating ovulation rate was 13 and 11 for Cal and NZW female rabbits respectively.

4-7-4-Fetus and embryonic mortality:

4-7-4-1-Implantation sites:

The obtained results set out in **Table 12** and **Fig. 26** shown that, the mean values numbers of implantation sites in NZW female rabbits were 8.33, 7.50 and 6.80 for control, 2nd and 3rd group respectively. From the results presented in **Table 12** and **Fig. 27** it can be observed that, there were decreased significantly differences ($P<0.05$) in number of implantation sites, due to the effect of copper oxychloride fungicide intoxication. The highest value in number of implantation sites in control group, which was 8.33, while the lowest value was 6.80 in the 3rd group (fed on 0.2368 gm Cu/kg. BW/day) for 60 days.

The obtained results showed that, number of implantation sites decreased when rabbits fed on concentration diets fungicide after 60 days. These results are in agreement with **keen *et al.* (1982)** who studied the effect of feeding diets high in copper metal to pregnant mice under varying condition, mice fed before and after the 5-days periods resulted in resorbtion frequency of more than 50%. And **El-Darawany *et al.* (1994)** who showed that rabbits when drank water contents heavy metals, suffered from low implantation sites.

Table 12: Least square means, standard error and percentage value for the effects of copper oxychloride fungicide on reproduction in pregnant doe rabbits.

Items	Groups		
	1st Group	2nd Group	3rd Group
No. of corpora lutea per female	8.67±0.21[b]	8.33±0.21[b]	9.67±0.21[a]
No. of implantation sites per female	8.33±0.32[a]	7.50±0.32[b]	6.80±0.32[c]
Post-implantation loss per female %	3.92 %[b]	9.96%[a]	29.68 %[a]

Means with the same letter within each row in each factor are non-significantly different ($P < 0.05$).
1st group (G_1) = Control,
2nd group (G_2)= (0.1184 gm Cu/kg. BW/day)
3rd group (G_3)= (0.2368 gm Cu/kg. BW/day)

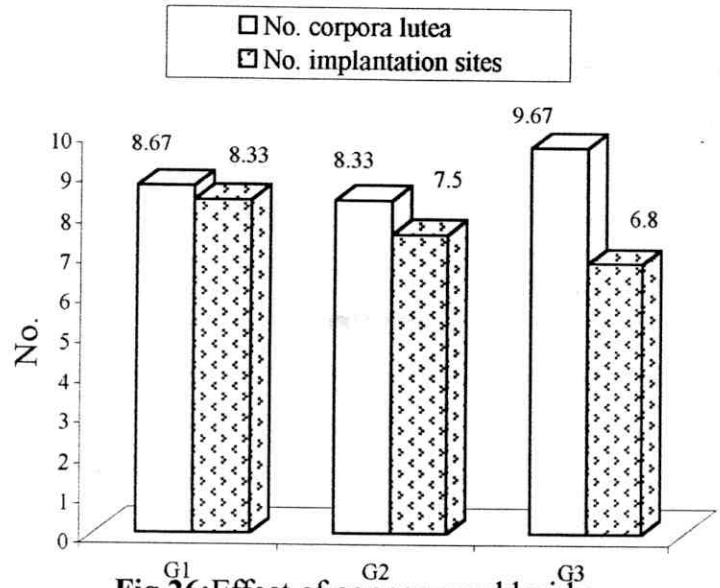

Fig 26: Effect of copper oxychloride fungicide on No. of corpora lutea and implantation sites.

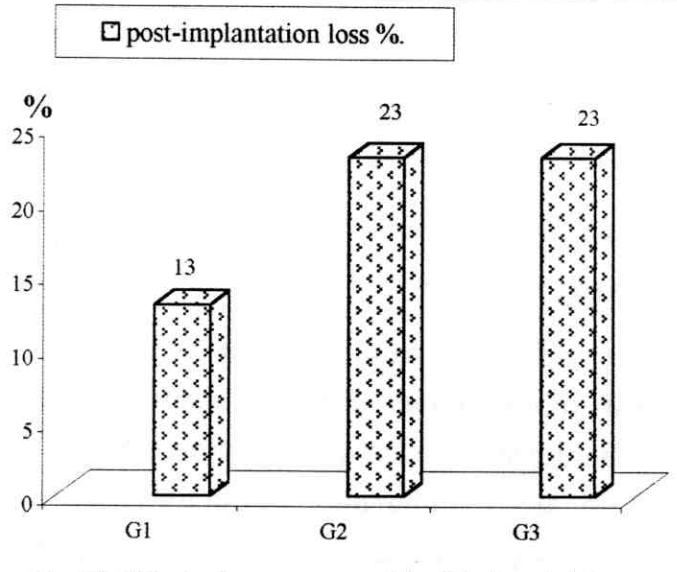

Fig 27:Effect of copper oxychloride fungicide on post-implantation loss.

4-7-4-2-Foetuses resorbtion:

From the obtained results presented in **Table 12** and **Fig.27,** the values percentage of post-implantation loss in NZW rabbits were 3.92 %, 9.96 % and 29.68 % in the 1st, 2nd and 3rd group respectively. These results were increased significantly differed ($P<0.05$) in post-implantation loss of NZW rabbits. The obtained results showed that, the post implantation loss was increased when female rabbits fed on contamination diets with copper oxychloride fungicide in **(Fig. 28, 29&30)** shown the normal emberyos in 1st group and resorbed foeti in both 2nd and 3rd groups respectively.

These results are in agreement with **El-Darawany *et al.* (1994)** who showed that pregnant female rabbits suffered from low implantation sites and the high percentage of resorbed foeti when drinking rabbits water contents a high copper and other heavy metals. And also with **Mariois and Bouvet (1972)** who showed that in rats when injection of copper from day 7 through 10 days of pregnancy resulted in a resorbtion frequency of 50 %.

4-7-5-Litter traits:

4-7-5-1-Litter size:

The average means of litter size at birth and weaning (28 days) in NZW rabbits under different treatment (feed copper oxychloride fungicide intoxication are presented in **Table 13** were 7.25, 5.75 and 5.25 bunnies at birth for control group, 2nd group (fed 0.1184 gm Cu/kg. BW/day) and 3rd group (fed 0.2368 gm Cu/kg. BW/day), while at the weaning the results were 6.25, 4.00 and 2.75 for the same groups respectively after 60 days.

Fig. 28: Uterine horn of NZW rabbits doe control group. indicate foetal normal development.

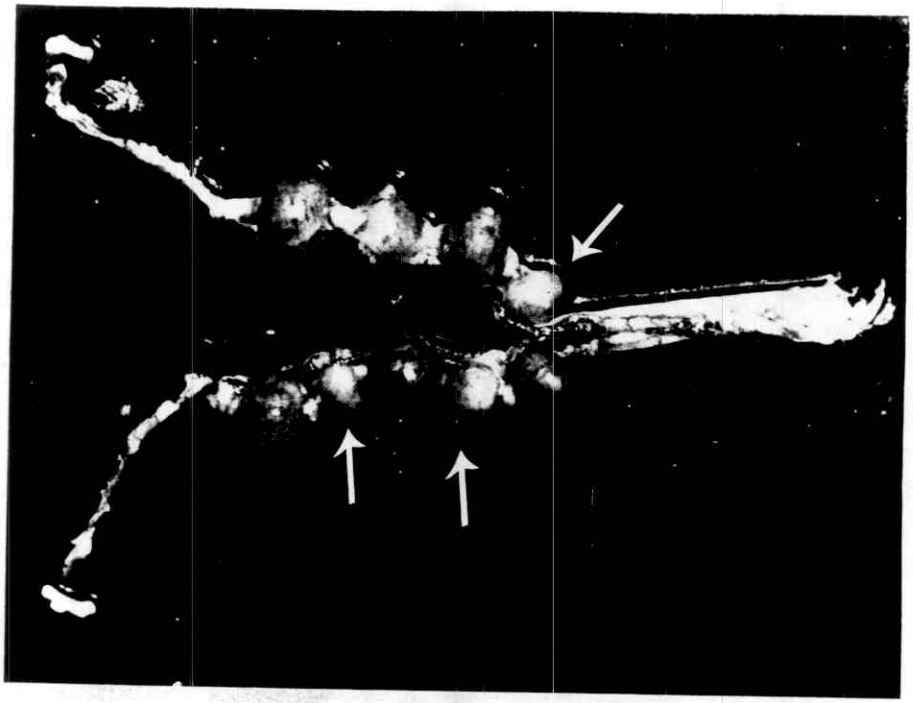

Fig. 29: Uterine horn of NZW doe rabbits fed on copper oxychloride (0.1184 gm Cu/kg. BW/day) as 1/20 LD_{50} for 60 days. Arrows indicate foetal resorbtion..

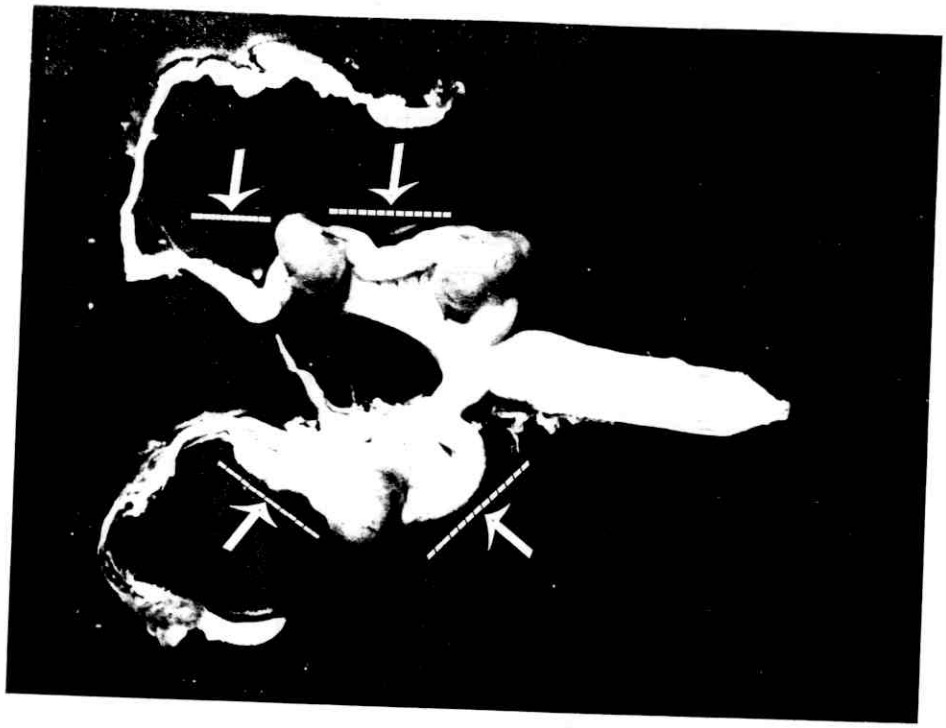

Fig.30: Uterine horn of NZW doe rabbits fed on copper oxychloride (0.2368 gm Cu/kg. BW/day) as 1/10 LD_{50} for 60 days. Showing foetal high resorbtion rate.

Table 13: Least square means, standard error and percentage value for the effects of copper intoxication on litter size and litter weight at (birth and weaning) and pre-weaning mortality in NZW rabbits.

Traits	No.	Litter size		Litter weight (gm)		Pre-weaning Mortality (%)
		at birth	at weaning (28 days)	at birth (gm)	at weaning (28 days) (gm)	
G_1: (Control)	5	7.25 ± 0.63^{a}	6.25 ± 0.39^{a}	371.25 ± 21.89^{a}	3385.0 ± 205.05^{a}	13.8 %^{c}
G_2: (1/20 LD50)	5	5.75 ± 0.63^{b}	4.00 ± 0.39^{b}	265.00 ± 21.89^{b}	1892.5 ± 205.05^{b}	30.4 %^{b}
G_3: (1/10 LD50)	5	5.25 ± 0.63^{b}	2.75 ± 0.39^{c}	172.50 ± 21.89^{c}	815.0 ± 205.05^{c}	47.6 %^{a}

Mean with the same letter within each column in each factor is non-significantly different ($P < 0.05$).
G_1= Control.
G_2= 0.1184 gm Cu/kg. BW/day.
G_3= 0.2368 gm Cu/kg. BW/day.

From the results obtained in **Tables 13&14** and **Fig. 31** it can be observed that, there were significant decreased ($P<0.05$) in litter size at birth and significant decreased ($P<0.001$) at weaning (28 days). The observed highest of value in litter size at birth which was 7.25 in control group while the lowest value of litter size at birth was 5.25 in 3rd group. The highest mean value in litter size at weaning (28 days) was 6.25 in control group while the lowest of mean value was 2.75 in the 3rd group.

From the results showed that, litter size at birth and at weaning were decreased when rabbits fed on diets copper oxychloride fungicide contamination. These results are in agreement with **Abdel-Samee and El-Masry (1992); Ahmed (1996) and Abdel-Rahman *et al.* (2000)** who reported that litter size at birth and at weaning in rabbits was decreased mineral concentrations in drinking water.

4-7-5-2-Litter weight:

The results presented in **Table 13** refer to litter weight at birth and at weaning (28 days) under exposured for copper oxychloride fungicide feed contamination. It could be seen from the table that, the means of litter weight at birth were 371.25, 265.00 and 172.50 gm in the control, 2nd (fed on 0.1184 gm Cu/kg. BW/day) and 3rd group (fed on 0.2368 gm Cu/kg. BW/day) respectively. And from the obtained results, the mean of litter weight at weaning was 3385.00, 1892.50 and 815.00 gm for the same groups respectively. These results were decreased significantly ($P<0.001$) in the litter weight at birth and at weaning (28 days) in **Table 14** and **Fig. 32**. The highest mean value of litter weight at birth and at weaning were 371.25 and

Table 14: F-ratio of analysis variance for the effect of copper intoxication on litter size and litter weight at (birth and weaning 28 days) and pre-weaning mortality in NZW rabbits.

S .O. V	df.	Litter size at		Litter weight(gm) at		Pre-weaining mortality
		birth	28 days	birth	28 days	
Group	2	5.68*	20.59***	20.99***	39.61***	0.27*
Error d.f	9					
Error MS		1.58	0.61	1915.97	168186.11	0.31

* = Significant ($P < 0.05$)
*** = Highly significant ($P < 0.001$)

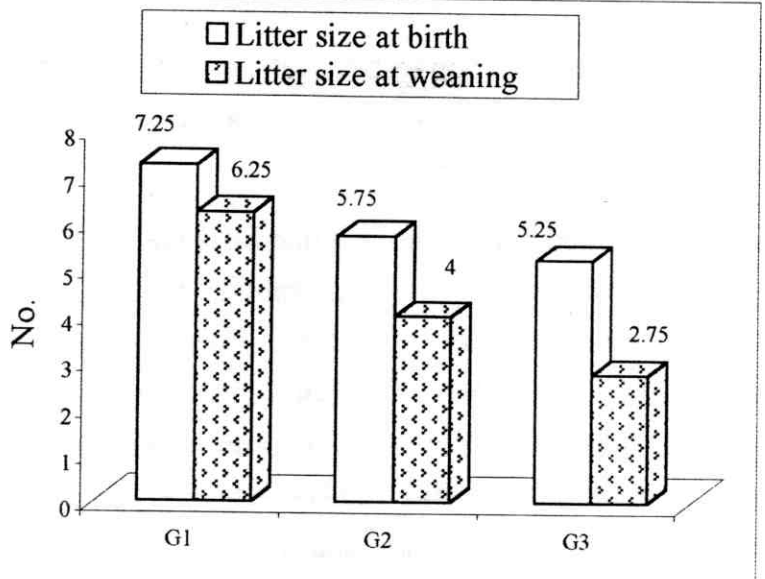

Fig 31:Effect of copper oxychloride fungicide on litter size at birth and at weaning

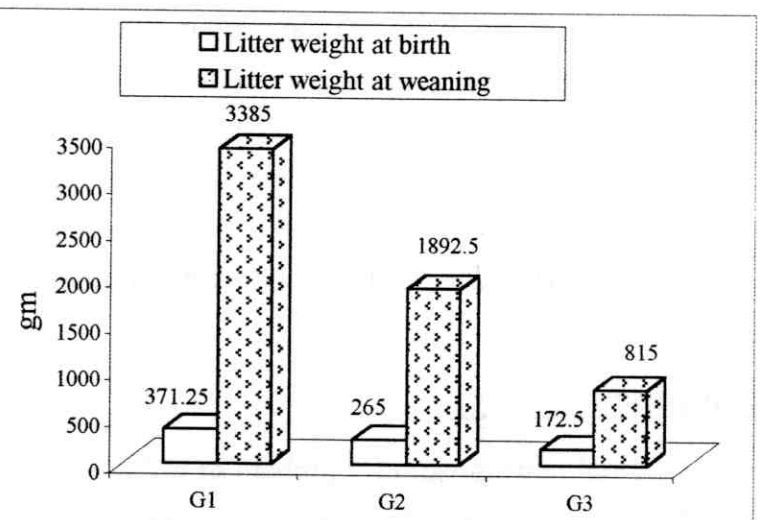

Fig 32:Effect of copper oxychloride fungicide on litter weight at birth and at weaning

3385.00 gm in the 1st group respectively, while the lowest mean value of litter weight at birth and at weaning were 172.50 and 815.00 gm respectively for 60 days.

The obtained results showed that litter weight at birth and at weaning was decreased when rabbits feeding of copper oxychloride fungicide contamination. These results are in agreement with **Borthakur *et al.* (2002)** who observed that, the least square means for litter weight at birth in NZW rabbits was 329.00 gm. And with, **Sedki (1991)** who showed that overall means of litter weight at birth and weaning were 318 gm and 1550 gm respectively.

4-7-5-3-Pre-weaning mortality rate:

The results presented in **Table13** and **Fig. 33** showed that pre-weaning mortality as exposure by copper oxychloride fungicide. It could be seen from the same table, that pre-weaning mortality percentages were 13.8 %, 30.4 % and 47.6 % in the 1st, 2nd and 3rd groups, respectively. These results increased significantly ($P<0.001$), which mortality rate was high in 3rd group. These results may be due to higher copper levels in milk, which transport from doe to their kids throughout lactation period.

4-8-Cytogenetic study:

The normal chromosome numbers of NZW rabbits were 44 chromosomes (2n=44). In this study, the microscopical examination indicated that, the mean values of total structural chromosomal aberrations in different groups, 1st (control), 2nd (treated with 1/20 LD_{50} fungicide as 0.1184 gm Cu/kg. BW/day)

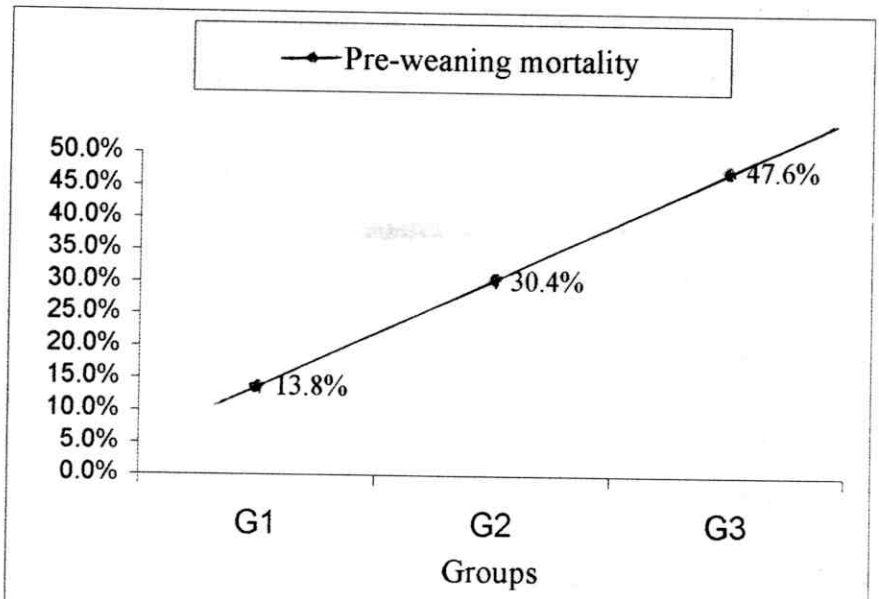

Fig.33: Effect of copper oxychloride fungicide intoxication on pre-weaning mortality rate.

and 3rd (treated with 1/10 LD_{50} fungicide as 0.2368 gm Cu/kg. BW/day) for 60 days were 2.71, 8.75 and 12.17 respectively in **Table 15**. These results increased showed significant ($P<0.001$) between groups. The obtained results in the same table revealed that the mean values between types (does and their offspring) were 9.33 and 6.42 respectively, it could be observed that, there were significant ($P<0.001$) for total structural chromosomal aberrations in **Table 16**. These results are in agreement with **Evering *et al.* (1991); Ahmed *et al.* (1998) and Moustafa (2000),** they attributed these changes to the genotoxic effect of the fungicide on chromosomes of rat bon marrow cells.

Fig. 34, 35, 36, 37, 38, 39, 40 and 41 showed the structure of normal chromosome and types of aberrations (gap, break, deletion, centromeric attenuation & centric fusion, end to end, ring and sticky) respectively.

4-8-1-Gaps and breaks:

The slide showed gap formation (small part in one chromatid was smaller than the width of the second chromatid) and chromosome break (the arm of one chromatid was smaller than its sister).

The mean values of gap chromosomes were 0.04, 0.38 and 1.42 in the 1st, 2nd and 3rd groups after 60 days from starting experiment, respectively in **Table 15**. The obtained results showed significant ($P<0.001$), while the mean values between types were 0.92 in mother (doe) rabbits and 0.31 in their offspring, the results could be observed that, there were significant differences ($P<0.01$) for gap in **Table 16.** These results agreed with **Bongso & Basrur (1976) and El-Nahass *et***

Table 15: Least square means and standard error for the effect of copper oxychloride fungicide on chromosomes of rabbits

Type of the treatment	No. of exam. animals	No. of Exam cells	Chromosomal aberrations								
			Structural aberrations								
			Gap	Break	Deletion	Centrom. att.	Centric fus.	End to end	Ring	Sticky	Total
Group:											
1st Group	12	600	0.04±0.18^{b}	0.13±0.16^{a}	0.83±0.3^{b}	0.38±0.22^{b}	0.54±0.19^{a}	0.33±0.13^{b}	0.17±0.2^{b}	0.25±0.2^{c}	02.71±0.5^{c}
2nd Group	12	600	0.38±0.18^{b}	0.42±0.16^{a}	2.92±0.3^{a}	0.88±0.22^{a}	0.63±0.19^{a}	0.13±0.13^{b}	0.88±0.2^{a}	1.63±0.2^{b}	08.75±0.5^{b}
3rd Group	12	600	1.42±0.18^{a}	0.42±0.16^{a}	2.88±0.3^{a}	1.38±0.22^{a}	0.88±0.19^{a}	0.58±0.13^{a}	1.21±0.2^{a}	2.17±0.2^{a}	12.17±0.5^{a}
Type:											
Doe	9	450	0.92±0.18^{a}	0.25±0.16^{a}	2.42±0.03^{a}	1.08±0.22^{a}	0.83±0.19^{a}	0.33±0.13^{a}	0.67±0.2^{a}	1.75±0.2^{a}	09.33±0.5^{a}
Offspring	27	1350	0.31±0.10^{b}	0.29±0.09^{a}	2.00±0.17^{a}	0.87±0.13^{a}	0.53±0.11^{a}	0.36±0.08^{a}	0.83±.0.1^{a}	0.94±0.1^{b}	06.42±0.3^{b}

Means with the same letter within each column in each aberration are non-significantly different ($P < 0.05$).
1st Group= control
2nd Group=0.1184 gm Cu/kg.BW
3rd Group=0.2368 gm Cu/kg.BW

Table 16: F-ratio of analysis of variance for effect of copper intoxication on structural chromosomes in NZW rabbits.

S.O.V.	df.	Gap	Break	Deletion	Centromeric attenuation	Centric fusion	End to end	Ring	Sticky	Total
Group (G) :	2	16.38^{***}	1.05	15.68^{***}	5.2^{**}	0.87	3.09^{*}	7.19^{**}	25.34^{***}	89.57^{***}
Type (T) :	1	8.92^{**}	0.54	1.44	2.71	2.03	0.03	0.53	12.63^{***}	24.91^{***}
GXT	2	6.87^{**}	0.67	1.29	0.43	0.07	1.46	0.31	3.17^{*}	5.99^{**}
Error df.	42									
Error MS		0.38	0.32	1.09	0.58	0.41	0.2	0.47	0.46	3.07

* =Significant ($P<0.05$).
** =Significant ($P<0.01$).
*** =Highly significant ($P<0.001$).

Fig. 34: A metaphase spread of NZW rabbits control group indicate normal chromosomes.

Fig. 35: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicate chromatid gap.

Fig. 36: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicates chromatid break.

Fig. 37: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicates chromatid deletion .

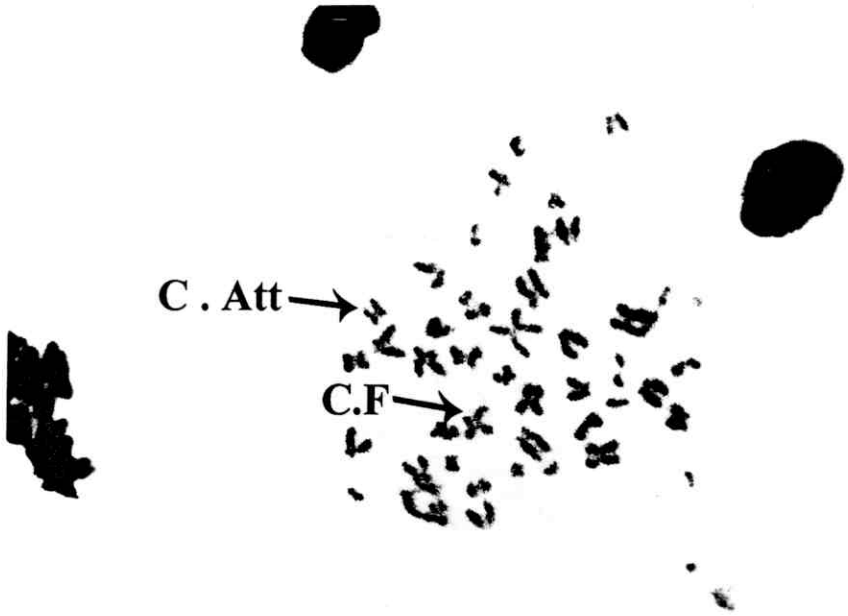

Fig. 38: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicates centromeric attenuation and centric fusion .

Fig. 39: A metaphase spread of NZW rabbits exposed to copper oxychloride Fungicide, arrow indicates end to end chromosomes.

Fig. 40: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicates ring chromosom.

Fig. 41: A metaphase spread of NZW rabbits exposed to copper oxychloride fungicide, arrow indicates sticky chromosomes.

al. **(1976).** They reported that. The gaps and breaks have been found reduced fertility in domestic animals. **Ahmed *et al.* (1998)** showed that, the effect of fungicide on induction of chromosomal aberrations in mature female albino rats bone marrow cells, the treatment induced significant increase ($P<0.01$) in percentage of chromosomal aberrations.

4-8-2-Deficiency or Deletion:

Deletion (one chromatid shorter than its sister chromatid). The obtained results showed that, the mean value of deletions were 0.83, 2.92 and 2.88 in the 1st, 2nd and 3rd groups after 60 days from starting experiment, respectively in **Table 15**. The observed results were increased significant ($P<0.001$) between treated groups compared with control group in **Table 16**. From the obtained results, the mean values of deletion chromosomes between types (does and their offspring) were 2.42 and 2.00 respectively. It could be noticed that, there were in significant differences. These results agreed with **Ahmed *et al.* (1998)**. They observed significant increase ($P<0.01$) when treated female albino rats by fungicide. Similar results were reported by **Ali (1992)** and **Fahmy (1995)**. They showed changes to the genotoxic effect of fungicide on chromosomes of mouse bone marrow cells. The mean values of deletion chromosomes were non-significant between groups and between types.

4-8-3-Centromeric attenuations:

The results showed in **Tables 15&16,** the mean values of centromeric attenuations cells were 0.38, 0.88 and 1.38 after 60 days from beginning of the experiment in the 1st, 2nd and 3rd groups, respectively. These results increased significantly

(P<0.01), and the high value was 1.38 in the 3rd group (fed on 0.2368 gm Cu/kg. BW/day) in aberrations, due to the effect of treatment. Similar results were reported by **Moustafa (2000)** who studied rat animals, **Ali (1992)** and **Fahmy (1995)** who showed that changes of the genotoxic effect of fungicide on chromosomes of mouse bone marrow cells

4-8-4-Centric fusion:

Centric fusion is the fusion of the centromeric regions of two acrocentric chromosomes, producing one meta- or submetacentric chromosome. The mean value of centric fusion cells were 0.54, 0.63 and 0.88 in 1st, 2nd and 3rd groups respectively. These results indicated that there were no significant differences between groups, The mean values of centric fusion between types were 0.83 for doe rabbits and 0.53 centric fusion chromosomes for their offspring, it could be observed that, there were non-significant. The obtained results disagreement with **Levan *et al.* (1972) and Gautam & Kpoor (1991)** on the bone marrow cells of mice treated with Dithan M-45 fungicide.

4-8-5-End to end association:

End to end association (two chromosomes were joined at proximal ends of the two chromatids).

Tables 15&16 indicated that the mean values of end to end association cells were 0.32, 0.13 and 0.58 in the 1st, 2nd and 3rd groups, respectively after feeding for 60 days. The obtained results increased significantly (P<0.05), and the high value in the 3rd group, due to the effect of treatment. These results are in

agreement with **Badawy (1995)** who found similar chromosomal abnormalities for rabbits. But the mean value between types were 0.33 for doe rabbits and 0.36 for their offspring, it could be observed that, there were non-significant.

4-8-6-Ring chromosomes:

The obtained results in Tables **15 &16**, showed that, the mean value of ring chromosomes were 0.17, 0.88 and 1.21 for the 1st, 2nd and 3rd groups after 60 days with treatment fungicide, respectively. These results increased significant differences ($P<0.01$) between groups and high value was in 3rd group, due to the effect of treatment.

These results are in agreement with the results obtained by **Ahmed *et al.* (1998).** They reported that, the percentage of ring chromosomes showed significant differences ($P<0.01$) when female rats were treated by fungicide, while the mean values between types were 0.67 for doe rabbits and 0.83 for their offspring, these results were no-significant.

4-8-7-Chromosome condensations (sticky):

Tables 15&16 showed that, the mean values of sticky chromosomes were 0.25, 1.63 and 2.17 in the 1st(control), 2nd (fed on 0.1184 gm Cu/kg. BW/day) and 3rd group (fed on 0.2368 gm Cu/kg. BW/day), respectively after feeding for 60 days. These results were increased significantly ($P<0.001$) between treated groups compared with control group. The high means value was 2.17 in 3rd group. From the results, the mean value was 1.75 sticky chromosomes for doe rabbits, while it was 0.94 for their offspring, the results were significantly between doe and

offspring rabbits. It could be noticed that due to the effect of treatment by fungicide.

These results are in agreement with the results obtained by many authors among them **Stancliffe & pirie (1971); Evans (1983); Rojik *et al.* (1983) and Hmdoon & Seddek (1995).** They reported that, the effect of pesticides range from the degeneration of the cell membranes up to disturbances of embryonic development and the observed frequencies of sticky chromosomal were almost about three times than that of the control in fish.