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EVALUATION OF THE TOXIC EFFECTS OF DIFENOCONAZOLE FUNGICIDE (SCORE) ON MALE ALBINO RATS

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Abstract

This study was designed to estimate the LD_{50} of Score (difenoconazole) fungicide and evaluate its toxic effects on body weight, liver weight, some biochemical parameters and hematological profiles of male albino rats after chronic oral daily doses ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ $LD._{50}$) for 8 weeks.

Rats were divided into 4 groups, the first group (G1) as control group, the other groups (G2, G3 and G4) treated daily with 20,40 and 80 mg/100 g.b.w. for 1, 4 and 8 weeks respectively

The medium lethal dose ($LD._{50}$) of Score was 400 mg /100g. body weight of adult male rat. Significant decreases in body weight, serum total protein, albumin, globulin, liver DNA and liver RNA, RBCs count, hemoglobin and blood Platelets count

However, significantly increase observed in hepatosomatic index, serum enzymes (ALAT, ASAT, ALP and CK), cholesterol, triglyceride, total lipids, serum creatinine and serum bilirubin and WBC's count

Keywords: Score, Enzymes, Protein, biochemical parameters, liver DNA, liver RNA and blood profile.

Introduction

Chemical pesticides are widely used in Egypt and other countries to minimize the loss of economic crops due to pest invading. Although, pesticides have been useful in pest control and plant disease, there is a considerable risk on human health **(Ergonen *et al*., 2005).**

wide range of compounds used as pesticide in agricultural and work as an agent to kill or control undesired pests, such as insects, weeds, rodents, fungi and other organism, which increases the food production (**Abhilash and Singh, 2009)** Wide spread use of pesticides in agriculture increases the intoxication to mammals **(Aktar *et al*., 2009).**

Score is a reliable, broad spectrum, systemic fungicide for long-lasting preventative and strong curative control including leaf spot diseases, powdery mildews, rusts and scab of annual and perennial crops. Score has excellent crop tolerance the crop stays healthy, vigorous and green which results in higher yields of better quality produce. Score is taken up by the plant and acts on the fungal pathogen during penetration and haustoria formation. It stops the development of

fungi by interfering with the biosynthesis of sterols in cell membranes. Although the mode of action permits protective and curative use, it is recommended to apply the product early enough to avoid irreversible crop damage and build-up of the disease

http://www.syngenta.com/country/eg/en/croppprptection/ourproducts/fungicides/Pages/Score250EC.aspx).

Fungicides are used agriculturally to control rust and mildew on fruit, vegetables, cereals and seeds, residential and commercial turf, and in pharmaceutical applications for the treatment of local and systemic fungal infections **(Goetz *et al*., 2007).** The fungicidal mode of action of triazoles involves disruption of fungal cell membranes and walls by the mechanism of inhibiting fungal lanosterol-14ademethylase, a xenobiotic metabolizing enzyme in fungi that has a homolog in mammals known as CYP51 **(Georgopapadakou, 1998 and Ghannoum and Rice, 1999),** which is evolutionarily conserved between plants, fungi, and animals. This enzyme is critical component for cholesterol synthesis and therefore steroide biosynthesis in animals **(Zarn *et al*., 2003).** Besides CYP51, triazoles also modulate the gene expression and enzyme activity of multiple cytochrome P450 (CYP) and other metabolic enzymes in mammalian liver and other tissues **(Ronis *et al*., 1994; Sun *et al*., 2005; Goetz *et al*., 2006 and Tully *et al*., 2006**). In spite of the massive use of these pesticides, only few developmental toxicological studies of azole fungicides have been published. As far as we know, there is no information dealing with the clastogenic or genotoxic effects of this agent on human or animal cells.

Fenarimol one of famous fungicide, may cause toxic effects by binding to cell macromolecules. **Di Ilio *et al*. (1995**) demonstrated the electrophilic nature of this fungicide and suggested its possible reactivity with DNA. When tested *in vivo* on rats, fenarimol was capable of inducing DNA damage in hepatocytes with a significant increase in DNA unwinding **(Grilli *et al*., 1991).** Fenarimol also induced prenatal and perinatal genotoxicity in leukocytes *in vivo* treated rats **(Castro *et al*., 2005).** Long-term bioassays analysing the carcinogenesis of fenarimol reported a significant increase in hepatic lesions (adenomas and hyperplasic nodules) in Wistar rats compared to untreated controls **(Ari and Dere, 2010).**

The present study is aimed to determine LD_{50} of the new fungicide (Score) and the biochemical and haematological effect on rats after treatment with 3 doses ($1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) were evaluated

Materials and Methods:

Chemicals

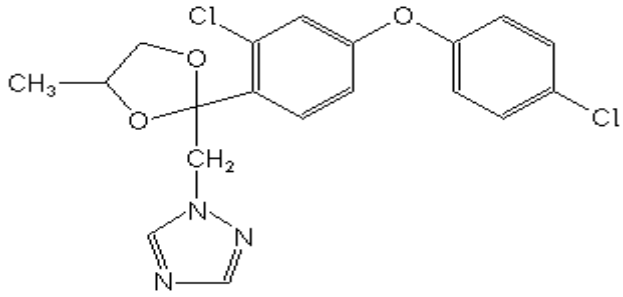
Fungicides used in this study namely; Score 250% EC (difenoconazole)

IUPAC:3-chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

Empirical Formula: $C_{19}H_{17}Cl_2N_3O_3$

Activity: Fungicides

***Structure*:**



Animals

Mature male albino rats, (*Rattus norvigicus*) weighting between 150–170 g.,they were brought from Helwan farm of Egyptian Organization for Vaccine and Biological preparation (vaccera). The animals were housed in the especially designed cage under good ventilation condition for one week before the experimental periods and were fed on Laboratory standard balanced diet manufactured by the Egyptian company for oil and soap as well as some vegetables as a source of vitamins.

Determination of LD.$_{50}$:

Forty two male rats were kept for one week prior of the experimentation for acclimatization. The rats were divided into 7 groups each group includes 6 rats were kept in a separated cages; the groups were divided to two categories. Group 1 was select as control and groups 2 to 7 treated with Score. The animals were observed after 24 hrs. and the dead animal was removed immediately, the LD.$_{50}$ was determined according to equation of **Behren and Karber (1953)**

$$LD._{50} = Dm - \frac{\sum Z.d}{M}$$

Where :

Dm = The higher dose used (first dose kill all animals in the group)

Z = The number of the dead animals of two successive doses divided by 2.

d = Difference between two successive dose.

$\sum$ = Total sum of (Z.d).

M = The number of animal in each group

General layout of the experiment

Experimental animals were divided into three groups as the following:

Group (G1): Consists of 18 normal healthy adult male albino rats served as control.

Group (G2): Consists of 18 normal healthy male albino rats treated with $^{1}/_{20}$LD.$_{50}$ chronic daily doses of score (20 mg/100 g. b. wt.) for 8 weeks

Group (G3): Consists of 18 normal healthy male albino rats treated with $^{1}/_{10}LD._{50}$ chronic daily doses of score (40 mg/100 g. b. wt.) for 8 weeks

Group (G4): Consists of 18 normal healthy male albino rats treated with $^{1}/_{5}LD._{50}$ chronic daily doses of score (80 mg/100 g. b. wt.) for 8 weeks Six animals from each group were sacrificed at intervals 1, 4 and 8 weeks.

Body and hepatosomatic index(HSI).

All animals were weighted before and after the end of each experimental period. Also, liver of each rat was isolated, weighted and stored in -20C^{o}. Their hepatosomatic index (HSI) was calculated as :

$$HSI = \frac{Li}{W} \times 100$$

Where

Li = Drained mass of liver (g).

W = The total body weight (g).

Sample collection

Blood samples were collected in two separate vials, one sample collected on EDTA for hematological studies, the other sample were collected without EDTA for preparation serum for biochemical analyses.

Methods:

Serum Alanine amino transferase enzyme (ALAT); Aspartate amino transferase enzyme (ASAT); Alkaline phosphatase enzyme (ALP) Lactate dehydrogenase enzyme (LDH); creatinine kinase enzyme (CK), serum glucose, total protein, albumin, globulin, total lipids, cholesterol and triglycerides**, c**reatinin and Bilirubine concentrations were estimated using Bio-Mérieux commercial Kits

RBCs and WBCs were counted by the hemocytometer method (**Stevens, 1997);** hemoglobin was determined by the cianometahemoglobin method according to **Lee**, ***et al.*** **(1998**); hematocrit was determined by the microhematocrit method **(Goldenfarb *et al.*,1971)**.

Liver tissues were prepared for DNA and RNA by washing with ice cold saline. One gram of these tissues were separated and homogenized in 5 ml. of ice-cold 0.1M phosphate buffer (pH 7.4) and centrifuged at 5000 g for 20 min. the particle free supernatant was used for estimation of DNA and RNA

DNA was estimated colormetrically according to the method of **Freifelder (1982)** and RNA was determined colormetrically by mans of the orcinol reaction according to **Dische (1937).**

Statistical Analysis:

The obtained data are expressed as mean ± standard error and the analysis was revised by **Quattro Pro for windows** program version 2- Microsoft Windows version 7. The significance of difference between the means was calculated according to student's t- test. Statistical significance was accepted at $P<0.05$ and $P<0.01$**.**

Results

The medium lethal dose ($LD_{.50}$) of Score was 400 mg /100 g. body weight of adult male rats (Table, 1)

Statistical analyses of the obtained data in table (2) showed significant decrease in body weight, serum total protein, albumin and globulin in all treated groups after 4 and 8 weeks of treatment with Score. While, the Their hepatosomatic index recorded significant increase for all experimental groups ($P\leq0.01$, $P\leq0.001$) after treatment for 4 and 8 weeks only when compared with control group.

The results in Table (3) showed the enzymes activity of ALAT, ASAT, ALP and CK after treatment with chronic daily doses (20,40 and 80 mg/ 100g. b.w.) of Score. The data showed significant increase ($p \leq 0.05$, $p \leq 0.01$) in the enzymes activity of ALAT, ASAT and ALP in all treated groups in all intervals (1, 4, and 8 weeks) when compared with control group.

Also, significant increase in the activity of serum LDH in all treated groups in all intervals except G2 after one week of treatment and after treatment with the previous doses of Score, the enzyme activity of serum CK showed significant increase ($p \leq 0.05$, $p \leq 0.01$) in all treated groups at all intervals except G2 and G3 after one week of treatment (Table, 3).

Statistical analyses of the obtained data in table (4) showed insignificant change in serum glucose level in all treated groups except after treatment with Score for 8 weeks show highly significant increase ($p \leq 0.01$). Also, the tabulated data showed significant increase ($p \leq 0.05$, $p \leq 0.01$) in levels of cholesterol, triglyceride, total lipids (Table, 4), creatinine and bilirubin in all treated groups after 1,4, and 8 weeks compared with control group (Table ,5).

In contrast, the recorded data in table (5) show significant decrease in liver DNA and liver RNA in all treated groups after 4 and 8 weeks of treatment with Score compared with control group

Data in table (6) show no significant changes in RBC´s count, WBC´s count, Hb and blood platelets count in all treated groups after one week of treatment with score at $^1/_{10}$, $^1/_{20}$ and $^1/_5$ mg/ 100 g. b. w. when compared with control group. Significant decrease in RBC´s count, Hb and blood platelets count in all treated groups with score at $^1/_{10}$, $^1/_{20}$ and $^1/_5$ mg/ 100 g. b. w. after 4 and 8 week of treatment. In contrast, the WBC´s count was significantly increased in all treated groups after 4 and 8 week of treatment with score at $^1/_{20}$, $^1/_{10}$ and $^1/_5$ mg/ 100 g. b. w. when compared with control group.

Table (1): Determination the medium lethal dose ($LD._{50}$) of Score fungicide in adult male rats after Behrnes & Karber (1953).

No. of animal group	Dose mg / 100 g b. wt. (Dm)	M	No. of animal death	d.mg	Z	Z.d	Mortality Rate %
1	0.0	6	0	0.0	0.0	0.0	0.0
2	300	6	1	50	0.5	25	16.6
3	350	6	3	50	2	100	50.0
4	400	6	3	50	3	150	50.0
5	450	6	4	50	3.5	175	66.6
6	500	6	4	50	4	200	66.6
7	550	6	6	50	5.0	250	100
						900	

$$LD._{50} = Dm - \frac{\sum Z.d}{M} = 550 - \frac{900}{6} = 400 \text{ mg / 100 g. body weight}$$

Where:

Dm = The higher dose used (first dose kill all animals in the group)

Z = The number of the dead animals of two successive doses divided by 2.

d = Difference between two successive dose.

$\sum$ = Total sum of (Z.d).

M = The number of animal in each group

Table (2): The effect of daily doses of Score fungicides ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) on body weight, hepatosomatic index, serum total protein, serum albumin and serum globulin of adult male albino rats after 1, 4 and 8 weeks of treatment

		Body weight (g.)	Hepatosomatic index (HSI)	Serum Total protein (g. %)	Serum Albumin (g. %)	Serum Globulin (g. %)
After 1 Week	G1	163.0±5.33	4.56± 0.15	7.80± 0.173	4.80±0.086	3.00± 0.166
	G2	162.5^{ns} ±3.13	4.88^{ns} ±0.11	7.01^{NS}± 0.208	4.10^{NS} ±0.114	2.91^{NS}± 0.132
	G3	166.3^{ns} ±4.15	4.55^{ns} ±0.09	7.11^{NS}± 0.183	4.11^{NS}±0.172	3.00^{NS}± 0.068
	G4	160.8^{ns} ±2.22	4.81^{ns} ±0.43	7.00^{NS}± 0.139	4.08^{NS}±0.055	2.92^{NS}±0.116
After 4 Week	G1	170.00±4.20	4.41±0.17	6.98± 0.159	3.78±0.068	3.20±0.128
	G2	159.34**±5.14	5.55*±0.07	6.10**± 0.118	3.38**±0.126	2.72**± 0.089
	G3	160.11**±3.72	5.59*±0.16	5.74**± 0.112	3.22**±0.156	2.52**± 0.107
	G4	148.31**±4.21	6.69**±0.22	5.44**± 0.093	3.22** ± 0.058	2.22**± 0.097
After 8 Week	G1	181.61±3.11	4.02±0.08	7.11± 0.216	4.00 ± 0.239	3.11± 0.051
	G2	161.41**±4.21	6.00**±0.23	6.14**± 0.254	3.26** ± 0.212	2.88**± 0.097
	G3	155.14**±5.10	6.44**±0.18	5.42**± 0.271	3.04** ±0.102	2.38** ± 0.183
	G4	143.3**±6.20	7.15**±0.33	5.16**± 0.136	2.88**±0.124	2.28**± 0.081

Table (3): The effect of daily doses of Score fungicides ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) on serum enzymes (ALT, AST, ALP, LDH and CK) in male rats after 1, 4 and 8 weeks of treatment

		ALAT (UI/l)	ASAT (UI/l)	APL (UI/l)	LDH (UI/l)	CK (UI/l)
After 1 Week	G1	20.50± 1.15	70.77± 2.26	91.21± 1.28	91.41±1.72	1699.7±177
	G2	24.22*± 1.14	78.19*± 0.47	103.14*± 3,06	96.15^{ns}± 2.33	1730.7 ^{ns} ±224
	G3	27.00*± 1.66	79.18*± 2,13	114.03**± 2.16	113.21*± 2.53	1727.7 ^{ns} ±213
	G4	33.35**± 1.57	80.11*± 2.90	121.00**± 3,79	123.04**± 4.06	1798.3*±187
After 4 Week	G1	23.05± 1.33	68.02± 2.21	90.21± 0.66	89.41± 0.99	1687.5±133
	G2	46.34**± 1.15	81.00**± 3.03	110.69*± 3,23	114.51**± 1,62	1787.0*±211
	G3	55.14**± 2.11	88.05**± 215	125.15**± 3.82	122.42**± 4.17	1796.4*±159
	G4	52.03**± 1.19	95.19**± 0.72	144.52**± 3.33	136.89**± 2.83	1825.4**±155
After 8 Week	G1	21.55± 2.13	67.07± 3.12	95.11± 1.09	89.11± 2.42	1690.2±185
	G2	71.38**± 0.99	102.11**±3.11	127.07**± 3.06	133.15**± 3.25	1820.3**±164
	G3	81.33**± 2.02	115.40**± 4.40	144,69**± 4.16	145.04**± 1,44	1815.2**±166
	G4	90.46**± 0.14	128.31**±3.66	151.43**± 2.33	155.00**± 2,31	1860.5**±206

Table (4): The effect of daily doses of Score fungicides ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) on serum glucose, cholesterol, triglyceride and total protein in male rats after 1, 4 and 8 weeks of treatment.

		Serum glucose (g/dl)	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum Total lipids (g/l)
After 1 Week	G1	80.13±2.22	2.20±0.137	82.53±1.36	0.840±0.111
	G2	81.05^{NS}±2.73	2.99**±0.30	99.60*±4.15	1.215*±0.168
	G3	81.22 ^{NS}±1.67	2.86**±0.18	134.10**±4.67	1.517*±0.192
	G4	84.11 ^{NS}±3.05	2.86**±0.15	151.30**±2.73	1.984**±0.241
After 4 Week	G1	82.22±3.34	1.97±0.13	85.44±1.85	0.860±0.130
	G2	83.95 ^{NS}±1.93	2.88**±0.17	133.07**±3.94	1.587**±0.154
	G3	87.00^{NS}±2.87	3.40**±0.15	148.10**±2.18	1.591**±0.186
	G4	85.52 ^{NS}±1.44	3.92**±0.24	155.70**±3.82	1.600**±0.154
After 8 Week	G1	84.43±2.83	2.01±0.11	86.1±1.81	0.820±0.112
	G2	139.23**±3.68	3.46**±0.88	129.66**±2.91	1.973**±0.151
	G3	148.33**±3.88	3.91**±0.13	148.32**±3.60	1.652**±0.163
	G4	155.93**±4.50	3.71**±0.79	161.61**±5.90	1.988**±0.177

Data are represented as means ± SE.; n = 6.
*** = p< 0.05, ** = p< 0.01 and ns = non significant compared to control**

Table (5): The effect of daily doses of Score fungicides ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) on serum creatinin , serum bilirubine, liver DNA and liver RNA in male rats after 1, 4 and 8 weeks of treatment

		Creatinin (mg/dl)	Bilirubine (Mmol/l)	DNA (µg/ mg wet weight of tissue)	RNA (µg/ mg wet weight of tissue)
After 1 Week	G1	0.71± 0.19	4.20±0.76	1.94 ± 0.43	3.21±0.13
	G2	0.98*±0.09	6.80**±0.41	1.81^{ns} ±0.21	3.09^{ns}± 0.24
	G3	1.09**±0.05	6.64**±0.85	1.78^{ns} ±0.47	3.11^{ns}± 0.08
	G4	1.07**±0.78	6.81**±0.06	1.80^{ns} ±0.48	3.01^{ns}± 0.57
After 4 Week	G1	0.69±0.32	4.24±0.63	1.86 ± 0.51	3.39± 0.22
	G2	0.94*±0.44	7.00**±0.69	1.64*±0.11	2.71*± 0.32
	G3	1.29**±0.56	8.25**±1.07	1.59*±0.09	2.35**± 0.41
	G4	1.42**±0.92	8.08**±1.79	1.44**±0.31	2.22**± 0.19
After 8 Week	G1	0.73±0.88	3.99±0.45	1.91 ± 0.55	3.00± 0.15
	G2	1.65**±0.80	8.74**±1.05	1.42**±0.37	2.36**± 0.71
	G3	1.75**±1.05	9.2**±0.72	1.28**±0.63	2.21**± 0.14
	G4	1.93**±0.11	10.71*±1.21	1.12**±0.33	1.91**± 0.08

Table (6): The effect of daily doses of Score fungicides ($^1/_{20}$, $^1/_{10}$ and $^1/_5$ LD_{50}) on R.B.Cs count, W.B.Cs count, Hb and blood Platelets count of adult male albino rats after 1, 4 and 8 weeks of treatment

		R.B.Cs count (X 10^6)	W.B.Cs count (X 10^3)	Hb (g/dl)	Platelets count
After 1 Week	G1	5.17±0.12	11.31±0.22	14.08±0.26	599.04±10.18
	G2	5.34^{NS}±0.04	11.67^{NS}±0.13	14.11^{NS}±0.50	611.70^{NS}±12.55
	G3	5.33^{NS}±0.11	12.10^{NS}±0.51	13.94^{NS}±0.23	610.74^{NS}±15.77
	G4	5.26^{NS}±0.14	11.88^{NS}±0.14	14.12^{NS}±0.21	609.61^{NS}±10.35
After 4 Week	G1	5.11±0.19	10.99±0.15	14.300±0.26	611.12±10.40
	G2	4.46*±0.39	11.98**±0.12	12.93**±0.24	521.07**±12.15
	G3	4.28**±0.16	13.23**±0.79	11.41**±0.27	511.33**±16.70
	G4	3.99**±0.09	14.44**±1.11	11.04**±0.18	517.33**±10.16
After 8 Week	G1	5.09 ±0.30	11.36±0.23	14.20±0.11	603.11±18.20
	G2	4.33**±0.54	13.79**±0.37	11.01**±0.13	501.04**±17.39
	G3	4.10**±0.28	14.91**±1.33	10.87**±0.33	479.00**±13.51
	G4	3.89**±0.12	15.30**±0.99	10.66**±0.25	462.05**±11.26

Data are represented as means ± SE.; n = 6.
*** = p< 0.05, ** = p< 0.01 and ns = non significant compared to control**

Discussion

The obtained data in table (2) showed significant decreases in body weight in all treated groups after 4 and 8 weeks of treatment with Score fungicides. This reduction in body weight may be probably occurred due to decreased food intake, food consumption and anemia cases of the treated animals. High rate of protein metabolism might be needed to fulfill energy requirements during detoxification with score fungicide. Probably, this may be another reason for weight loss in animals exposed to Score fungicides in the present study. This seems to be in agreement with **Joshi *et al.* (2005) and Ananthan and Kumaran (2013)** where they found that, significantly decreased ($p < 0.05$) in body weight after administration of Mancozeb at dose of 300mg/kg body weight for 60 days.

While, the hepatosomatic index recorded significant increases for all experimental groups ($P \leq 0.01$, $P \leq 0.001$) after treatment for 4 and 8 weeks only when compared to control group. The increases in HSI values in the present data was referred to the accumulation of fungicides, these results were suggested by many authors **(Kurutas *et al.*, 2006; Abdel-Hameid, 2007 and Jelodar and Fazli, 2012).** In contrast, several investigations have recorded decreases in hepatosomatic index of different experimental animals under the effect of various insecticides **(Chung *et al*., 2002 and Kalender *et al*., 2006).**

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism **(Rehulka and Parova, 2000 and Li *et al*., 2010).** Serum enzymes AST, ALT LDH and CK are frequently used to determine the toxic effects of various pollutants **(Li *et al*., 2011).**

Obtained data in table (2) showed significant decreases in serum total protein, serum albumin and serum globulin in all treated groups after 4 and 8 weeks of treatment with Score fungicides when compared to control group. This decrease could be due to a decrease in the rate of protein synthesis or as a result of chronic disease when large number of parenchyma liver cells has been destroyed. Furthermore; the decrease of total serum protein may caused by the reduction of serum globulin level which markedly declined at the same time. This was accompanied by a decrease in body weight gain which led to body weight loss at the end of the experiment **(Baron, 1984 and Zama *et al*., 2006)**

Similar results were recorded by **Mohssen (2000)** and **Baligar and Kaliwal (2001),** they reported that mancozeb fungicide at doses of 600, 700 and 800 mg/kg/day induced a significant decrease in the level of protein content in rats. Also, **Al-Amoudi (2012)** showed significant decrease in the level of protein after orally treated with metalaxyl fungicide at dose level of $^{1}/_{10}$ LD_{50} (130 mg/kg body weight) three times per week for 4 continuous weeks.

Our result were disagreement with **Basir *et al.* (2011),** who showed increased in serum total proteins and serum albumin in rabbits after intoxicated with lambda-cyhalothrin fungicide.

The tabulated data in Table (3) revealed a significant increases in the enzymes activities of serum ALAT, ASAT and ALP after treated with chronic daily doses (45,90 and 180 mg/ 100g. b.w.) of Score fungicide in all treated groups at all intervals (1, 4, and 8 weeks) when compare with control group. These Elevations in theses enzymes may be attributed to acute hepatocellular damage or extrahepatic obstruction, or both. This result is in agreement with **Antonelli *et al*. (2007); Sakr (2007) and Sakr and Al-amoudi (2012)**; they reported that oral administration of mancozeb fungicide to male rats induced changes in the activities of ALT and AST throughout the period of the study in a dose dependent manner.

Sukul and Spiteller (2000) reported that the fungicide bithionol sulfoxide cause hepatotoxicity, including an increase in serum AST, at high doses (50, 500 and 1000 mg/kg). Also, oral administration of male rats with mancozeb (500, 1000 and 1500 mg/kg/day) for 90, 180 and 360 days induced changes in the activities of ALT and AST throughout the period of treatment **(Takaori, 1993; Sakr and Lamfon, 2005; Al-Amoudi, 2012 and Waghe *et al*., 2013).**

The elevated creatine kinase (CK) activity together with higher LDH activity indicates that there was damage to other peripheral tissues as well. Usually, LDH and CK indicate muscle damage. CK elevation is usually indicative of myositis or myocardial damage. Creatine kinase is a catalyst of ATP renewal in peripheral tissues under anaerobic conditions, and LDH in peripheral tissues turns pyruvate into lactate to compensate ATP generation under diminished oxidative phosphorylation. Thus, elevation in those two enzymes (CK and LDH) also points that there was a hypoxic condition because of fungicides treatment **(Edge *et al*., 2006 and El-Sayed *et al*., 2007).**

A significant increase in serum glucose, cholesterol, triglyceride levels and total lipid were recorded in all groups after treated with Score fungicide (Table, 4). Theses increased in serum glucose levels were agreement with **Veerappan *et al*. (2011)** who reported significant increases of serum glucose levels at 50mM dose of carbendazim administered to rats and for all durations. The increase in glucose level may be attributed to the disruption of glucose intake and use by cells **(WHO / IPCS, 1993; Selmanoglu *et al*., 2001 and Borges *et al*., 2007).**

The previous increase may be caused by one of the following two major mechanisms solely or by their combination; the first type is associated with raised levels of plasma fatty acids resulting from the mobilization of fat from adipose tissue and the second type is usually due to a metabolic blockade in the production of plasma lipoprotein which may be come from one mechanism or more, blockade in apoprotein synthesis, blockade in synthesis of the lipoprotein from lipid, apoprotein failure in provision of phospholipids found in lipoprotein and failure in the secretory mechanism itself **(Terada *et al*., 1998)**

Similar findings were found by **Sakr and Abel-Samie (2008),** they reported that acute thiram (tetramethyl-bis-thiocarbamyl disulphide) poisoning caused decreased lipoprotein lipase (LPL) activity in adipose tissue and increased the levels of total plasma cholesterol, triacylglycerols and high density lipoprotein (HDL) cholesterol

in the affected rats. Moreover, **Reena-Kackar *et al*. (1999)** speculated that treating mice with metalaxyl increased the degree of tissue lipogenesis and that this increase was most likely achieved through the acceleration of acetyl-CoA, which is known to be the precursor of cholesterol biosynthesis.

The present results revealed also a significant increase in serum creatinine in response to Score toxicity. These increases in creatinine might be due to impaired kidney function by the used fungicide. This was supported by **Kluwe (1981)** who indicated that an elevation of creatinine level in the blood is an indicative of impaired kidney function. Similar results were obtained by **El- Shenawy *et al*. (2009)** in mice and **Sarhan and. Al-Sahhaf (2011)** in rabbit. They proved that treatment rabbit with diazinon (20 mg/kg body weight, every 48 hrs) for 4 weeks induced significant increases in serum creatinine. The obtained results were disagreement with **Ahmed (2006)**, who reported that no significant effect of Diazinon fungicide ($^{1}/_{30}$ LD50) on creatinine level.

The present study was revealed that prolonged exposure of Score fungicide caused decrease in the levels of liver DNA and RNA in all treated group after 4 and 8 weeks, these results agree with **Castro *et al*. (2005) and Ksheerasagar and Kaliwal (2006).**

However, these decreases in the levels of liver DNA and RNA might be caused as results of genotoxic action by decreased mitotic index and disturbed cell division **(Topaktas *et al*., 1996)** or due to inhibitory action of Score fungicide on DNA and RNA synthesis **(Walter *et al*., 1980)** or by cell death due to focal necrosis **(Shivanandappa and Krishnakumari, 1981).**

Di Ilio *et al*. (1995) has demonstrated the electrophilic nature of the fungicide and suggested its possible reactivity with DNA. When tested in vivo in rats, fenarimol was capable of inducing DNA damage in hepatocytes with a significant increase in DNA unwinding **(Grilli *et al*., 1991)** and a reduction in mitotic index at higher doses in mice **(Aydemir and Bilaloglu, 2004).**

The data in table (6) show that there was a gradual decrease in erythrocyte count, haemoglobin content and the number of blood platelets in rats treated with score fungicide. After 4 and 8 weeks of treatment, this decrease was statistically significant. While, the leukocyte count was significantly increased . These results are in agreement with those obtained from previous studies of the haematological effects of fungicides on mammalian animals, **Al-Amoudi (2012)** reported that after treating mice with metalaxyl for dosage of $^{1}/_{10}$ LD50 (which was equivalent to 130 mg/kg body weight) at a rate of three times per week for 2 and 4 weeks induced a significant decrease in the RBC count (5.2±0.24), haemoglobin content (11.5±2.3) and the number of blood platelets (543±15.3). While, metalaxyl treatment induced a significant increase in the WBC count (5.8±0.4).

The decrease in the RBC count and haemoglobin content in the present study was indicated that Score fungicides cause anaemic. This anaemia my be due to one of the two reason, (a) increased blood as a result of the accelerated red cell

destruction by haemolytic agents or rapid cell removal from an abnormality of cell shape or over-activity of the spleen (b) a quantitative decrease in blood formation as a result of a quantitative decrease in red marrow from aplasia or a quantitative decrease in marrow activity from a deficiency in the substances necessary for normal bone marrow activity (**Mehadevaswami *et al.*, 2001)**.

In contrast**, Basir *et al.* (2011)** show that a blood analysis of rabbits treated with lambda-cyhalothrin revealed a significant decrease in RBC count, and WBC counts, haemoglobin concentration and lymphocytes, while mean corpuscular haemoglobin concentration, mean corpuscular volume, neutrophils, monocytes and eosinophils showed significant increased **(Akhtar *et al.*, 2009).**

References

1. **Abdel-Hameid, N. H. (2007):** Physiological and Histopathological Alterations Induced by Phenol Exposure in *Oreochromis aureus* Juveniles. Turkish Journal of Fisheries and Aquatic Sciences, 7: 131-138.
2. **Abhilash, P.C. and Singh, N. (2009):** Pesticide use and application: An Indian scenario. J Hazardous Mater. 165: 1-12.
3. **Ahmed. S. K. (2006):** hepatic and renal biochemical responses to the toxicological interaction between acetylsalicylic acid and diazinon in albino rats. J. Egypt. Soc. Toxicol., 35: 1-6.
4. **Aktar, W.; Sengupta, D. and Chowdhury, A. (2009):** Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2(1): 1–12.
5. **Akhtar, N.; Srivastava, M.K. and Raizada, R.B. (2009):** Assessment of chlorpyrifos toxicity on certain organs in rat, Rattus norvegicus. Journal of Environmental Biology, 30(6): 1047-1053
6. **Al-Amoudi, W. M. (2012):** Haematological and Biochemical Effects of Metalaxyl Fungicide on Albino Mice American Journal of Biochemistry, 2(5): 62-66.
7. **Ananthan, G. and Kumaran, B. (2013):** Effect of Mancozeb on the Specific activities of Testicular Phosphatases and Protective role of Vitamin C in Albino rats. Bulletin of Environment, Pharmacology and Life Sciences Bull. Env. Pharmacol. Life Sci., 2(7):56-61.
8. **Antonelli, A.C.; Torres, G.A.S.; Soares, P.C.; Mori, C.S.; Sucupira, M.C.A. and Ortolani, E.L. (2007):** Ammonia poisoning causes muscular but not liver damage in cattle. Arq. Bras. Med. Vet. Zootec., 59 :8–13.
9. **Ari, F. and Dere, E. (2010):** Effect of the sterol demethylation –inhibiting fungicide Fenarimol on selected biochemical parameters in rats. Acta Veterinaria (Beograd), 60 (1): 31-38.
10. **Aydemir, N. and Bilaloglu, R. (2004):** The investigation of the genotoxic effects of fenarimol and propamocarb in mouse bone marrow in vivo, Toxicol Lett., 28:73-78.
11. **Baligar, P.N. and Kaliwal, B.B. (2001):** Induction of gonadal toxicity to female rats after chronic exposure to mancozeb, Ind. Health, 39: 235-243.
12. **Baron, D. N. (1984):** A short textbook of chemical pathology.ELBS,5thed. The English language book society and holder and Stoughton, pp.188-206.

13. **Basir, A.; Khan, A.; Mustafa, R.; Khan, M. Z.; Rizvi, F.; Mahmood, F. and Yousaf, A. (2011):** Toxicopathological effects of lambda-cyhalothrin in female rabbits (Oryctolagus cuniculus)".Hum Exp Toxicol., 30 (7): 591-602.

14. **Behrens, S. and Karber, J. (1953):** Determination of LD_{50}. Arch.For Experienta. Pharmacol, 3: 177-372.

15. **Borges, V. C.; Rocha, J.B.T.; Savegnago, L. and Nogueira, C.W. (2007):** Repeated administration of diphenyl ditelluride induces hematological disorders in rats, Food Chem Toxicol., 45: 1453-1458.

16. **Castro, V.; Mello, M.; Poli, P. and Zucchi T. (2005):** Prenatal and perinatal fenarimol induced genotoxicity in leukocytes of in vivo treated rats, Mutat Res., 583, 95-104.

17. **Chung, J.; Kalman, D.; Moore, L.; Kosnett, M.; Arroyo, A.; Beeris, M.; Mazumder, D. and Smith, A. (2002):** Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. Environ. Health Perspect., 110:729–733.

18. **Di Ilio, C.; Sacchetta, P.; Iannarelli, V. and Aceto, A. (1995):** Binding of pesticides to alpha, mu and pi class glutathione transferase, Toxicol Lett., 76: 173-177.

19. **Dische, Z. (1937):** Mit dem Hauptoxy dereduktinosprozess der Blutglycolyse gekoppelte synthese der Adenosintriphosphor saure. Enzymologia, 1:228-310.

20. **El-Sayed, Y.S.; Saad, T.T. and El-Bahr, S.M. (2007):** Acute in - toxication of deltamethrin in monosex Nile tilapia, Oreochromis niloticus with special reference to the clinical, biochemical and haematological effects. Environmental Toxicology and Pharmacology, 24, 212–217.

21. **El-Shenawy, N. S.; Al-Eisa, R. A.; El-Salmy, F. and Salah, O. (2009):** Prophylactic effect of vitamin E against hepatotoxicity, nephrotoxicity, haematological induces and histopathology induced by diazinon insecticide in mice, Curr. Zool., 55 (3): 219-226.

22. **Edge, K.; Chinoy, H. and Cooper, R. (2006):** Serum alanine aminotransferase elevations correlate with serum creatine phosphokinase in myiositis. Rheumatology, 45:487–488.

23. **Ergonen, A.T.; Salacin, S. and Ozdemir, H. (2005):** Pesticides use among greenhouse works in Turkey. J. Clin. Foren. Med., 12:205-208.

24. **Freifelder, D. (1982):** Physical Biochemistry: Applications to Biology, W. H. Freeman and Company, New York, New York.

25. **Georgopapadakou, N. H. (1998):** Antifungals: Mechanism of action and resistance, established and novel drugs. Curr. Opin. Microbiol., 1: 547-557.

26. **Ghannoum, M. and Rice, L. (1999):** Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin. Microbiol. Rev., 12: 501–517.

27. **Goetz, A. K.; Bao, W.; Ren, H.; Schmid, J. E.; Tully, D. B.; Wood, C. R.; Rockett, J. C.; Narotsky, M. G.; Sun, G.; Lambert, G. R. and *et al*. (2006):** Gene expression profiling in the liver of CD-1 mice to characterize the hepatotoxicity of triazole fungicides. Toxicol. Appl. Pharmacol., 215: 274–284.

28. **Goetz, A. K.; Ren, H.; Schmid, J. E.; Blystone, Ch. R.; Thillainadarajah, I.; Best, D. S.; Nichols, H. P.; Strader, L. F.; Wolf, D. C.; Narotsky, M. G.; Rockett, J. C. and**

Dix, D. J. (2007): Disruption of Testosterone Homeostasis as a Mode of Action for the Reproductive Toxicity of Triazole Fungicides in the Male Rat. Toxicol. Sci., 95(1): 227–239

29. **Grilli, S.; Ancora, G.; Rani, P.; Valenti, A.M.; Mazzullo, M. and Colacci, A. (1991):** In vivo unwinding fluorimetric assay as evidence of the damage induced by fenarimol and DNOC in rat liver DNA, J Toxicol Environ Health., 34: 485-94.

30. **Goldenfarb, P.B.; Bowyer, F.P. Hall; T. and Brosious, E. (1971):** Reproducibility in the hematology laboratory: the microhematocrit determination, Am. J. Clin. Pathol. 56: 35–39.

31. **Jelodar, H. T. and Fazli, H. (2012):** Monthly Changes in Condition, Hepatosomatic Index and Bioavailability in Frogs (*Rana ridibunda*). Research Journal of Biology, 2 (1): 9-14.

32. **Joshi, S.C., Gulati, N. and Gajraj, A. (2005):** Evaluation of toxic impacts of mancozeb on testis in rats. Asian J. Exp. Sci., 19 (1): 73 – 83.

33. **Kalender, S.; Kalender, Y.; Ates, A.; Yel, M.; Olcay, E. and Candan, S. (2002):** Protective role of antioxidant vitamin E and catechin on idarubicin-induced cardiotoxicity in rats. Braz. J. Med. Biol. Res., 35: 1379-1387

34. **Kluwe, W. (1981):** Renal function tests as indicators of kidney injury in subacute toxicity. Toxicol. Appl. Pharmacol., 57: 414-424.

35. **Ksheerasagar, R. L. and Kaliwal, B. B. (2006):** Histological and Biochemical Changes in the Liver of Albino Mice on Exposure to Insecticide, Carbosulfan. Caspian J. Env. Sci., 4(1):67-70.

36. **Kurutas,E. B.; Doran, F. and Çıralık, H. (2006):** The effect of Endosulfan on lacticdehydrogenase enzyme system in liver of Mus musculus:A histochemical study. Eur J Gen Med., 3(4):148-151

37. **Lee, R.G.; Foerster, J.; Jukens, J.; Paraskevas, F.; Greer, J.P. and Rodgers, G.M. (1998):** Wintrobe's—Clinical Hematology (10th ed.), Lippincott Williams & Wilkins, New York, USA 2763 p.

38. **Li, Z.H.; Velisek, J.; Zlabek, V.; Grabic, R.; Machova, J.; Kolarova, J. and Randak, T. (2010):** Hepatic antioxidant status and hematological parameters in rainbow trout, Oncorhynchus mykiss, after chronic exposure to carbamazepine. Chemico-Biological Interactions, 183:98-104.

39. **Mehadevaswami, M. P.; Jardaramkunti, U. C.; Hiremath, M. B. and Kaliwal, B. B. (2001):** Effect of mancozeb on ovarian compensatory hypertrophy and biochemical constituents in hemicastrated albino rat" Reprod. Toxicol., 14 (2): 127-137.

40. **Mohssen, M. (2000):** Biochemical and histopathological changes in serum creatinine and kidney induced by inhalation of Thimet (Phorate) in male Swiss albino mouse, *Mus musculus*. Environmental Res., 87 (1):31-36.

41. **Reena-Kackar, E.; Srivastava, M. K.; Raizada, R. B. and Kackar, R. (1999):** Assessment of toxicological effects of mancozeb in male rats after chronic exposure" Indian J. Exp. Bio., 37(6): 553-559.

42. **Rehulka, J. and Parova, J. (2000):** Effects of diets with different lipid and protein contents on some blood and condition indices of rainbow trout, Oncorhynchus mykiss (Walbaum). J. Anim. Sci.,45:263-269.

43. **Ronis, M. J.; Ingelman-Sundberg, M. and Badger, T. M. (1994):** Induction, suppression and inhibition of multiple hepatic cytochrome P450 isozymes in the male rat and bobwhite quail (Colinus virginianus) by ergosterol biosynthesis inhibiting fungicides (EBIFs). Biochem. Pharmacol., 48: 1953–1965.

44. **Sakr, S.A. (2007):** Ameliorative effect of ginger (*Zingiber officinale*) on mancozeb fungicide induced liver injury in albino rats. Australian J. Basic Appl. Sci., 1(14): 650-656.

45. **Sakr, S. A. and Abel-Samie, H. A. (2008):** Apoptosis related protein Bax in liver of metalaxyl fungicide –treated mice: The effect of antox" Ozean J. Appl. Science, 1(1): 17-27.

46. **Sakr, S.A. and Al-amoudi, W.M. (2012):** Protective effect of silymarin on metiram fungicide-induced hepatotoxicity in albino rats. Research J. of Pharmaceutical, Biological and Chemical Sciences, 3 (1): 691-699.

47. **Sakr, S.A. and Lamfon, H.A. (2005):** Effect of green tea on metalaxyl fungicide induced liver injury in albino mice. Oxford Res. Forum J., 2(2): 65-69.

48. **Sarhan, O.M.M. and Al-Sahhaf, Z.Y. (2011):** Histological and Biochemical Effects of Diazinon on Liver and Kidney of Rabbits. Life Sci. J., 8 (4) 1183-1159.

49. **Saxena, P. and Saxena, A. K. (2010):** Cypermethrin Induced Biochemical Alterations in the Blood of Albino Rats. Jordan Journal of Biological Sciences, 3(3): 111-114.

50. **Selmanoglu, G.; Barlas, N.; Songur, S. and KocSkaya, E.A. (2001):** Carbendazim induced haematological, biochemical and histopathological changes to the liver and kidney of male rats, Hum Exp Toxicol., 20:625-30.

51. **Shivanandappa, T. and Krishnakumari, M. K. (1981):** Histochemical and biochemical change sin rats fed dietary benzene hexachloride. Ind. J. Exptl. Biol., 19: 1163- 1168.

52. **Stevens, M.L. (1997):** Fundamentals of Clinical Hematology, W.B. Saunders, Philadelphia, PA.

53. **Sukul, P. and Spiteller, M. (2000):** Metalaxyl: persistence, degradation, metabolism, and analytical methods" Rev Environ Contam Toxicol., 164:1-26.

54. **Sun, G.; Thai, S.; Tully, D. B.; Lambert, G. R.; Goetz, A. K.; Wolf, D. C.; Dix, D. J. and Nesnow, S. (2005):** Propiconazole-induced cytochrome P450 gene expression and enzymatic activities in rat and mouse liver. Toxicol. Lett., 155: 277–287.

55. **Takaori, H. (1993):** Thiophanate-methyl combined chronic toxicity/ oncogenicity study in rats" Unpublished report No. RD-9327 from Nisso Institute for Life Sciences, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

56. **Topktas, M.; Renci Zogullari, E. and Ila, H. B. (1996):** In vivo chromosomal aberrations in bone marrow cells of rats with marshal. Myt. Res., 371: 259-264.

57. **Tully, D. B.; Bao,W.; Goetz, A. K.; Ren, H.; Schmid, J. E.; Strader, L. F.; Wood, C. R.; Best, D. S.; Narotsky, M. G.; Wolf, D. C., Rockett, J.C. and Dix, D.J. (2006):** Gene expression profiling in liver and testis of rat to characterize the toxicity of triazole fungicides. Toxicol. Appl. Pharmacol., 215: 260–273.

58. **Veerappan, M.; Pandurangan, M.; Suriyamurthy, M. and Srikumar, K. (2011):** Acute Toxicological Evaluation of Low Dose Methyl 2-benzimidazole Carbamate Fungicide on Male Albino Rats. Iran J. of toxicology, 4 (4): 381-389

59. **Waghe, P. S.; Saini, S.; Rampal, N.; Prakash, P. and Lokesh, L. V. (2013):** Sub-Chronic Exposure to Carbendazim Induces Biochemical and Hematological Alterations in Male Goats. Toxicological & Environmental Chemistry, 95 (2): 330-336.

60. **WHO/IPCS. (1993):** Carbendazim. Environ Health Criter Monogr, 149:1-17.

61. **Zarn, J.; Bruschweiler, B. and Schlatter, J. (2003):** Azole fungicides affect Mammalian steroidogenesis by inhibiting sterol 14 alphademethylase and aromatase. Environ. Health Perspect., 111: 255– 261.

http://www.syngenta.com/country/eg/en/croppr ptection/ourproducts/fungicides/Pages/Score250EC.aspx).