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BIOCHEMICAL STUDIES ON RESIDUES OF TWO DIFFERENT FORMULATIONS OF PROFENOFOS INSECTICIDE IN *Oreochromis niloticus*

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ABSTRACT

The present study was undertaken to investigate the deleterious effects of sublethal concentrations of two commercial formulations of profenofos (selecron and cord) on haemogram, liver, and kidney functions and hepatic lipid peroxidation in *O. niloticus* fish .The results revealed a significant decrease in erythrocyte counts (RBCs), haemoglobin content (Hb) and mean corpuscular haemoglobin concentration (MCHC)at 0.566 mg/l of selecron, whereas a significant increase in packed cell volume (PCV), mean corpuscular volume (MCV)and mean corpuscular haemoglobin (MCH) at both concentrations of selecron after 7 days of exposure. There was a significant reducing in RBCs counts, Hb content and MCHC values at the higher concentration of selecron (0.850 mg/l) and a significant elevation in PCV, MCV, and MCH was detected for 14 days as well as this trend was noticed in leukocyte counts (WBC_s) at both treatments of selecron after 14 days of exposure. During the recovery period, the values of these parameters did not return to the control level. With respect to cord insecticide, there was a significant decline in RBCs counts, Hb content, PCV and MCHC at 0.390 mg/l for 7 days, however a significant increase in WBCs count was observed at both concentrations of cord for 14 days of exposure. But within the recovery period, these values returned to the control level. At 14 days, the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were elevated markedly in selecron-exposed fish. This elevation was continued during the recovery period. As for cord insecticide, there was a significant rise in AST activity at both treatmentsfor 7 days and at lower concentration after 14 days of

treatment as well as ALT activity was elevated markedly at 0.390 mg/l.for 7 days of exposure. The urea level was elevated significantly at the higher concentration of two tested formulations for 7 days and at the higher concentration of selecron (0.850 mg/l.) for 14 days. Also a significant enhancement in creatinine level was observed at both treatments of selecron during the experimental period (21 days). In cord treated fish, a significant increase in creatinine level was detected at 0.390 mg/l.for 7 days and at both concentrations during the rest periods of experiment. The hepatic malondialdehyde MDA levels was elevated significantly at 0.566 mg/l. of selecron for 14 days and within the recovery period and a significant decrease in malondialdehyde MDA level was noticed at 0.234 mg/l of cord formulation for 14 days and restore to the control level during the recovery period. The high residue levels of the two formulations in different organs and muscle flesh of *O. niloticus* fish were detected in liver. In contrast, the low residue levels in both formulations were detected in brain fish with all treatments. Furthermore, the higher persistent rate of profenofos in both formulations in water was detected at zero time and after 24 hours of application. While, the percentage losses of profenofos in both cord and selecron formulations were increased by lapse of time .The rate of persistent of cord formulation was lower than selecron formulation in water after 14 days of application at low and high concentrations used. Also, the identified of the different components of the two formulations used was achieved by using GC/MS.
Key words: Profenofos - *Oreochromis niloticus* .

INTRODUCTION

Because of the variety of human activities, the aquatic environment is becoming increasingly threatened by xenobiotics. Many of them may have deleterious effects which could be enhanced by a bioaccumulation (e.g. heavy metals or pesticides). Organophosphate (OP) has replaced organochlorine pesticides because of their rapid breakdown in water and their low environmental persistence. However, these insecticides used in intensive agricultural production can reach the aquatic environment either via seapage of chemicals from the soil or directly due to the spraying against pests (Castillo *et al.,* 1997). In addition, these compounds may become concentrated in the organs of aquatic

organisms, especially those at the top of the food chain. Profenofos commonly know as one of the organophosphate widely used for the control of agricultural pests in Egypt. Several publications revealed the existence of pesticide residues in various aquatic ecosystems were studied by several investigators (Miny and Sastry, 1989; Hassan *et al.*, 1996; Badawy, 1998; El-Kabbany *et al.*, 2002 and Radwan and Atalla 2005). The different components within two different formulations were identified by using IR, and GC/MS analysis.

MATERIALS AND METHODS

1) Experimental animals:

Healthy of fresh water *O. niloticus* (weight 82.05±6.34 g, length 13.6±0.43 cm.)Purchased from the farm of the Central Research of Fish Laboratory- Abbasa, Sharkia governorate and broughs to laboratory where acclimatized for 15 day under laboratory conditions. Physicochemical characteristics of the used water were analyzed pH (7.44±0.048), Temperature (21.44±0.79°C), Electrical conductivity (342.601±2.292 um ho/cm), Salinity (0.10±0.001 ppt) and Total hardness (229.58±3.93 mmol/l as $CaCO_3$). Feeding was continued (1.5% B.wt) over the course of the studies.

2) Chemicals:

Profenofos formulated as selecron and cord formulations (72% EC), which obtained from Syngenta-Switzeraland and Helb Chemical and Pesticides Company, Egypt, respectively.

Common name: profenofos

Trade names: selecron and cord

Chemical name: O-(4-bromo 2-chlorophenyl)-O-ethyl S-propyl phosphorothioate

3) Experimental procedure:

A static acute toxicity bioassay was performed according to standard method (OECD, 1984) to determine the 96-h. LC_{50}.value of selecron (72% EC.) and cord (72% EC.) Formulations for *O niloticus*. The toxic effects of selecron and cord for *O. niloticus* was evaluated according to the protocol of Fish Prolonged Toxicity Test: 14- days study. (OECD, 1992). The fish was divided into two groups of 12 each. Group one, were exposed to sub-lethal concentrations of tested formulations either selecron or cord and which represented half of 96h.-LC_{50} and group two was exposed to the safe threshold

concentrations of tested formulations. In addition, a third group serves as control group. After 14 days of exposure; fish were removed in tanks with clean water free from insecticide for 7 days as recovery period. The blood samples were taken out from caudal vein by hepranized syringe for haematological and biochemical analyses after 7 and 14 days of profenofos exposure as well as during the recovery period. On each sampling, five fish were removed from the holding aquaria and the vital organisms such as liver, kidney, brain, spleen, heart, gills and muscle were removed for residues analysis.

4) Haematological analysis:

The blood samples were collected in tubes containing the EDTA (ethylene diamintetra acetic acid) (1 mg/1ml blood), for counting the RBCs and WBCs by using the method of Blaxhall and Daisly (1973). Also, The Hb and PCV were determined and the erythrocytes indices calculated by using the values of RBCS, Hb and PCV, according to the methods of (Jain, 1986).

5) Biochemical analysis:

Plasma samples were obtained after centrifugation of blood (10 min at 2000 xg) and then stored at -20°C until biochemical analysis. The biochemical tests including liver enzymes, viz, alanine and aspartate amino- transferases (ALT & AST) activities, renal function parameters such as urea and creatinine. All biochemical parameters were determined by using laboratory commercial kit. The aminotransferases (ALT & AST) activities were determined according to the method of Reitman and Frankel (1957). The urea and creatinine were measured by using the method of Couloumb and Fareau (1963) and Husdan and Raroport (1968), respectively.

6) Determination of lipid peroxidation in hepatic tissue:

The end products of polyunsaturated fatty acid peroxidation were determined as thiobarbituric acid reactive substances (TBARS) in whole homogenate of hepatocytes of treated and control fish according to the method of Ohkawa *et al.* (1979). The values of (TBARS) were expressed in terms of malondialdehyde (MDA) n mol/g wet tissue.

7) Identification of the finger print and additive compounds which found in selecron and cord formulations:

7.1) Infra red (IR):

The finger print of profenofos appeared from the Infra Red instrument (KBr) Vmax/Cm-1 (AVATAR 330 FT-IR Thermo Nicolet).

7.2) GC/MS analysis of selecron and cord formulations:

The additive compounds within cord and selecron formulations were subjected to GC/MS analysis using GC conditions injection temperature 250°C-Oven temperature program 180-250°C (5°C /min) Mass Spectroscopy (MS) was recorded in the electron impact (E.I) mode at 70 ev. The Reconstructed Ion Chromatogram (RIC) of mass spectra of peaks for selecron and cord along with a computerized library search for the identify of each peak.

8) Residues analysis:

8.1) Extraction of profenofos insecticide:

Fish samples (1 g) of liver, kidney, brain, spleen, and heart with 10ml of acetone and 100ml of acetone to (50 g) fish muscle flesh and gills were added and blended in warring blended at high speed centrifuge for 2 min and partition with dichloromethane (Mills *et al.*, 1972) .

8.2) Water samples:

Residues levels of profenofos in cord and selecron formulations in water were determined at zero, 24, 48, 72, 96, 120 h., 6, 7 and 14 days after application. Extraction of profenofos residues from water samples were done according to the method of Mann (1981). Water samples 100ml. of each treatment were transferred into conical flask and 200ml of dichloromethane were added and then the samples were shaken mechanically using electrical shaker for 1hour. The organic layer was separated in separatory funnel and was evaporated till dryness.

8.3) Clean up:

The resulting extracts of fish tissues were cleaned by activated florisil using elution solvent system of 50% dichloromethane, 48.5%n-hexane and 1.5% acetonitril (Mills *et al.*, 1972) .The pesticide extracts were evaporated at 30°C to dryness. After clean up the profenofos extract dissolved in 1ml methanol to HPLC analysis with UV detector and C18 stainless column 25 mm. The HPLC conditions for profenofos, impurity as 4-bromo 2-chlorophenyl and biperonyl butoxide as synergist were recorded on table (1).

Table (1): The conditions for the determination of profenofos,4-bromo 2-chlorophenyl and biperonyl butoxide by high performance liquid chromatography (HPLC).

Chemical compounds	Mobile phase	Flow rate ml/min	Retention time Rt	Detection limit µ /kg
Profenofos	Methanol80/acetontryl15/ H_2O5	1	3.277	0.06
4-bromo 2-chlorophenyl	Methanol90/acetonitrile10	0.9	3.175	0.05
Biperonyl butoxide	Methanol90/acetontryl10	0.9	3.504	0.05

Analysis of profenofos, 4-bromo 2- chlorophenyl, and biperonyl butoxide was carried out with HPLC. Duplicate injection (2ul.)Of calibration solution and each sample were injected and integrated areas for each peak were recorded and standard peak under Ideal condition in Fig. (1) For profenofos, Fig. (2) for 4-bromo 2-chlorophenyl and Fig (3) for biperonyl butoxide.

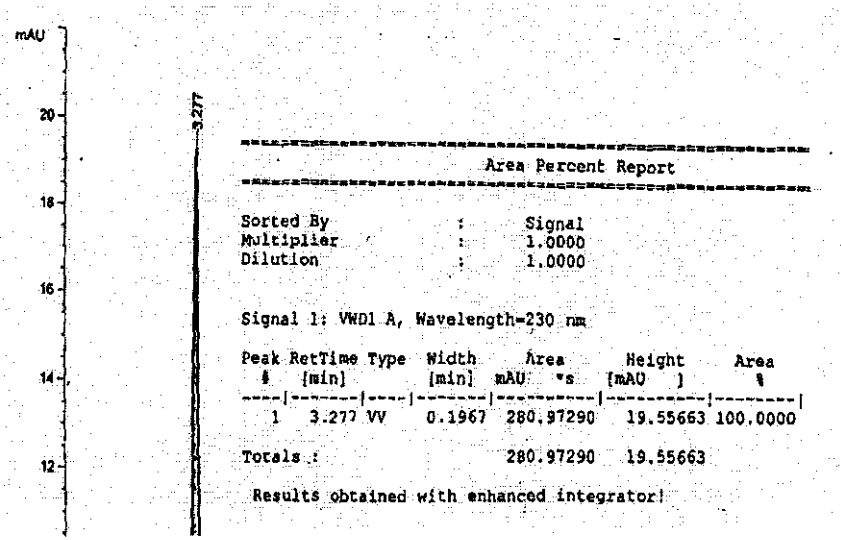

Figure (1): Chromatogram of profenofos on HPLC

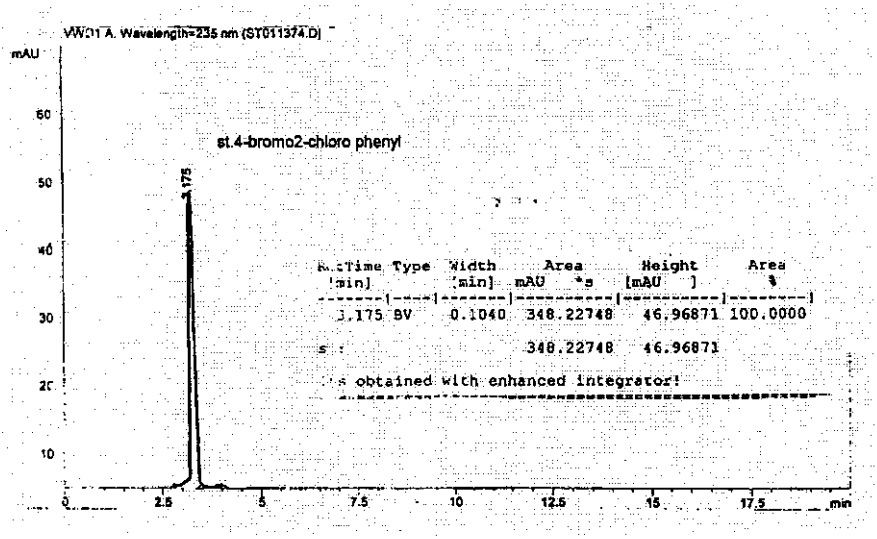

Figure (2): Chromatogram of 4-bromo 2- chlorophenyl on HPLC

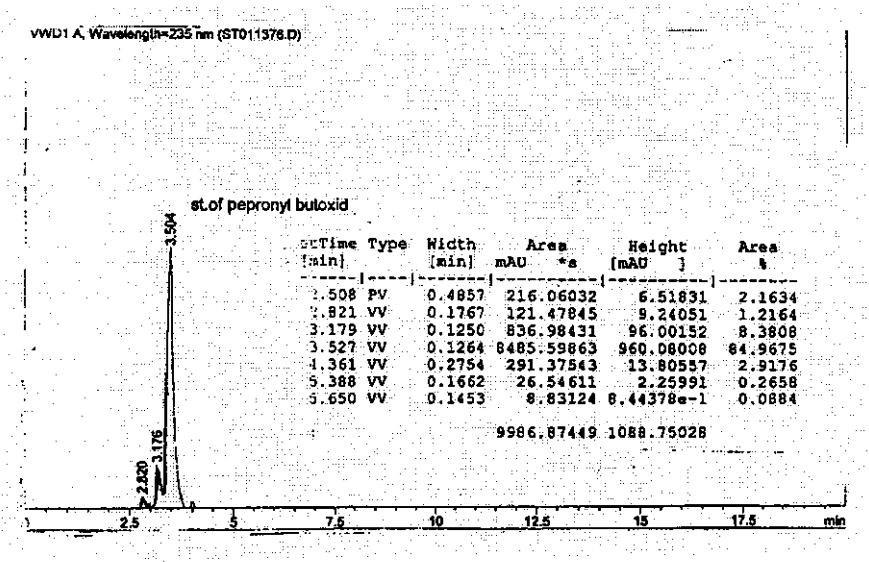

Figure (3): Chromatogram of biperonyl butoxide on HPLC

9) Statistical treatment of the results:

Results are expressed as mean ± Standard Error (SE.) the statistical significant of the difference between control and insecticide treated fish by the students "T" test (Gad &Weil 1989).

RESULTS AND DISCUSSION

1) Acute toxicity test:

The 96-h.LC_{50} (median lethal concentration) of selecron and cord formulations, for *Orieochromis niloticus* was estimated to be 1.74 and 0.78 mg/l., respectively. The cord formulation proved to be more toxic to *O. niloticus* than selecron formulation, this may be attributed to existence of biperonyl butoxide in cord formulation which inhibits detoxication process by binding pipronyl butoxide metabolites to cytochrome P450 enzymes, thus preventing the enzymes from detoxifying pesticide in hepatocytes of *O. niloticus* (Hodgson and Levi, 1998). Biperonyl butoxide considered moderately acutely toxic to fish. LC_{50}:3.94 -6.12 mg/l (Osmitz and Hobson, 1998).

2) Prolonged toxicity test (14 days) :

2.1) Haematological and biochemical effects of selecron and cord formulations:

Haematological results are listed in (Tables, 2 & 3).A significant decrease in the erythrocyte counts (RBCS), haemoglobin content (Hb) and mean corpuscular haemoglobin concentration (MCHC)was observed in *O. niloticus* following to selecron at 0.566 and 0.850 mg/l after 7 and 14days (Table, 2). While, a significant increase in the total leukocyte count (WBCS), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemogolbin (MCH) recorded at the lower concentration of selecron for 7 days. Also, this trend were noticed in PCV, MCV and MCH parameters at the higher concentration (0.850 mg/l) after 7 days of treatment .In addition, there was a significant decline in the RBCS, Hb and MCHC at 0.850 mg/l of selecron after 14 days of treatment. Whereas, a significant elevation in the values of PCV, MCV and MCH observed Also a significant increase in the WBCS counts at both concentrations of selecron for 14 days was observed. The haematological changes were permanent during the recovery period in selecron- exposed fish. The results in Table 3, show that the cord treatments caused a

Table (2): Effect of different concentrations of selecron (profenofos) on haemogram in *O.niloticus*

Time / Parameter	7 days			14 Days			Recovery for 7 day		
	Control	0.566 mg/L	0.850 mg/L	Control	0.566 mg/L	0.850 mg/L	Control	0.566 mg/L	0.850 mg/L
RBCs ($x10^6/\mu L$)	1.014 ± 0.127	0.608 ± 0.904*	0.715 ± 0.135	1.043 ± 0.125	0.988 ± 0.103	0.531 ± 0.089*	0.965 ± 0.033	0.758 ± 0.032	0.406 ± 0.030***
WBCs ($x10^3/\mu L$)	6.343 ± 0.283	11.725 ± 1.688	8.863 ± 2.354	5.816 ± 0.401	14.716 ± 2.515*	13.530 ± 0.939***	7.021 ± 0.263	8.873 ± 1.059	13.200 ± 0.834***
Hb (g/dl)	5.058 ± 0.127	0.608 ± 0.049*	5.69 ± 0.670	5.50 ± 0.194	4.406 ± 0.598	3.893 ± 0.414**	5.358 ± 0.158	4.743 ± 0.359	3.597 ± 0.436**
PCV (%)	19.333 ± 0.182	27.74 ± 1.50***	21.884 ± 0.171***	19.00 ± 0.547	20.524 ± 2.650	22.50 ± 2.73***	19.666 ± 0.795	21.666 ± 0.912	21.670 ± 1.825
MCV (Ft)	243.130 ± 41.878	422.73 ± 30.696*	499.313 ± 17.942**	296.226 ± 19.83	273.642 ± 47.096	362.935 ± 3.815***	273.340 ± 16.163	277.776 ± 49.024	533.32 ± 29.688***
MCH (pg)	34.676 ± 1.544	66.168 ± 10.717*	96.560 ± 7.237***	39.932 ± 3.418	38.259 ± 4.653	80.111 ± 9.728**	33.821 ± 2.196	46.269 ± 6.67	86.874 ± 4.377***
MCHC (%)	27.485 ± 0.046	17.508 ± 4.309	25.019 ± 1.927	27.633 ± 2.71	31.604 ± 4.080	17.188 ± 0.036**	27.333 ± 0.393	22.028 ± 2.789	16.798 ± 2.489***

Values Shown are mean ± S.E.

*P<0.05 **P<.01 ***P< 0.001

Table (3): Effect of different concentrations of cord (profenofos) on haemogram in *O.niloticus*

Time / Parameter	7 days			14 Days			Recovery for 7 day		
	Control	0.234 mg/L	0.390 mg/L	Control	0.234 mg/L	0.390 mg/L	Control	0.234 mg/L	0.390 mg/L
RBcs x $10^6/\mu$L	1.014 ± 0.127	0.753 ± 0.058	0.483 ± 0.073**	1.043 ± 0.125	0.915 ± 0.015	0.835 ± 0.0162	0.965 ± 0.033	1.470 ± 0.052	0.989 ± 0.188
WBcs x $10^3/\mu$L	6.343 ± 0.283	19.10 ± 0.774***	10.073 ± 1.287***	5.816 ± 0.401	13.273 ± 0.370***	15.106 ± 0.607***	7.021 ± 0.263	9.423 ± 1.101	6.416 ± 1.085
Hb g/dl	5.058 ± 0.127	4.949 ± 0.755	1.385 ± 0.136***	5.50 ± 0.194	6.812 ± 0.280	4.159 ± 0.382	5.356 ± 0.156	5.777 ± 0.334	4.541 ± 0.938
PCV %	19.333 ± 0.182	18.127 ± 0.849	15.666 ± 0.182***	19.00 ± 0.547	19.333 ± 0.966	19.00 ± 3.209	19.666 ± 0.795	20.00 ± 0.632	21.666 ± 0.483
MCV ft	243.136 ± 41.878	209.799 ± 16.149	386.043 ± 91.349	266.176 ± 19.83	224.789 ± 21.886	269.471 ± 52.452	261.017 ± 25.457	216.899 ± 15.835	263.623 ± 39.947
MCH pg	34.676 ± 1.544	33.103 ± 4.237	29.371 ± 4.448	39.932 ± 3.418	48.08 ± 6.588**	52.010 ± 8.675	33.821 ± 2.196	39.265 ± 2.533	41.840 ± 3.634
MCHC%	27.485 ± 0.046	25.738 ± 2.958	8.918 ± 0.987***	27.633 ± 2.71	33.029 ± 1.437	32.059 ± 7.195	27.333 ± 0.393	28.996 ± 1.761	32.803 ± 0.139

Values shown are mean ± S.E.

* P< 0.05 ** P < 0.01 *** P < 0.001

significant decrease in RBCs counts, Hb content, PCV values and MCHC in *O.niloticus at* 0.390 mg/l for 7 days, whereas a significant elevation in the counts of WBCs was noticed at both concentrations of tested formulation after 7 and 14 days of exposure. The leukocytesis disappeared in cord treated fish during the recovery period (Table, 3). Biochemical measurements in selecron-exposed fish, showed a significant enhancement in the activity ALT at higher concentration of selecron (0.850 mg/l) after 7 days and also at both concentrations of selecron after 14 days. With respect of AST, a significant elevation in the activity of AST was noted at 0.566mg/l for 14 days and a significant inhibition in this parameter was detected at the higher concentration of selecron (0.850 mg/l) for 14 days. This elevation in the activities of aminotransferases (AST & ALT) was continued throughout the recovery period with concerning of cord treatments, a significant elevation in the activity of ALT was observed only at the higher concentration of cord after 7 days of treatment (Table, 4). Also, a significant increase in the activity of AST was noticed at both concentrations after 7 days and at higher concentration after 14 days and restored to the control level during the recovery period. As for renal function, there was a significant elevation in the levels of urea at 0.850 mg/l of selecron after 7 and 14 days of exposure and within the recovery period, the urea level returned to the control values Also, there was a significant increase in the levels of creatinine for 7 and 14 days of treatment as well as during the recovery period. (Table, 5). The results in Table 5 exhibit that the urea level was elevated markedly only at 0.390 mg/l for 7 days. However, the creatinine level also was increased markedly at 0.390 mg/l and at both tested concentrations after 7 and 14 days of exposure, respectively Also during the recovery period, the creatinine level was elevated markedly when compared with control group (Table, 5).The concentration of malondialdehyde (MDA) was elevated markedly in the whole homogenate of liver of fish following exposure to lower concentration of selecron insecticide this may be due to weak of stimulation of detoxification process in selecron-exposed fish. Where no significant differences in MDA level was observed in fish exposed to higher concentration of selecron in compared with control fish after 14 day of exposure, this in turn, suggestive of an adaptive response to selecron insecticide at higher concentration of selecron insecticide. In contrast, the level of MDA was declined significantly in cord- treated fish with

Table (4): Effect of different concentrations of selecron (profenofos) on liver enzymes and renal functions in *O.niloticus*

Time / Parameter	7 days			14 Days			Recovery for 7 day		
	Control	0.566 mg/L	0.850 mg/L	Control	0.566 mg/L	0.850 mg/L	Control	0.566 mg/L	0.850 mg/L
ALT activity U/L	25.645± 0.835	23.416± 1.304	70.444 ± 4.329***	24.850± 1.916	76.333 ± 5.251**	83.875± 0.039**	28.25 ± 1.579	89.666± 1.688***	57.962± 4.135***
AST activity U/L	87.693 ± 6.741	91.718± 10.341	76.482± 6.409	84.934± 2.616	170.879± 8.146**	63.849± 2.741**	96.794± 5.301	163.332± 8.587**	99.866± 23.577
Urea mg/dL	3.676± 0.632	4.352 ± 0.145	5.344 ± 0.346*	3.853 ± 0.222	3.292 ± 0.502	7.353 ± 1.188*	3.277 ± 0.501	2.096 ± 0.278	2.968 ± 0.387
Creatinine mg/dL	0.141 ± 0.014	0.346 ± 0.036**	0.538 ± 0.052 ***	0.168 ± 0.018	0.346 ± 0.031**	0.614 ± 0.021***	0.161 ± 0.011	0.384 ± 0.041***	0.557 ± 0.038**

Values shown are mean ± S.E.

* P< 0.05 ** P < 0.01 *** P < 0.001

Table (5): Effect of different concentrations of cord (profenofos) on liver enzymes and renal functions in *O.niloticus*

Time / Parameter	7 days			14 Days			Recovery for 7 day		
	Control	0.234 mg/L	0.390 mg/L	Control	0.234 mg/L	0.390 mg/L	Control	0.234 mg/L	0.390 mg/L
ALT activity U/L	25.645 ± 0.835	52.30 ± 10.32	68.05 ± 2.984**	24.85 ± 1.916	32.50 ± 3.402	30.20 ± 3.893	28.25 ± 1.579	27.80 ± 0.863	34.083 ± 2.806
AST activity U/L	87.693 ± 6.741	130.060 ± 2.617**	150.838 ± 3.482**	84.934 ± 2.616	147.481 ± 9.686**	95.104 ± 9.366	96.794 ± 5.301	71.345 ± 3.080***	106.434 ± 12.637
Urea mg/dL	3.676 ± 0.672	4.064 ± 0.107	6.289 ± 0.572**	3.853 ± 0.222	4.296 ± 0.189	4.385 ± 0.619	3.277 ± 0.501	4.222 ± 0.162	4.606 ± 0.318
Creatinine mg/dL	0.141 ± 0.014	0.153 ± 0.020	0.346 ± 0.036***	0.168 ± 0.018	0.474 ± 0.060**	0.441 ± 0.037**	0.161 ± 0.011	0.317 ± 0.042*	0.441 ± 0.037***

Values shown are mean ± S.E.

* P< 0.05 ** P < 0.01 *** P < 0.001

lower concentration whereas during therecovery period the MDA level resumed to control level this may be attributed to enhancement of excretion of lipid peroxidative products in fish following exposure to lower concentrations of cord (Table, 6). It was established that exposure to toxicants produce many haematological and biochemical changes in fish which precede cellular systemic dysfunction. When the fish, *O. niloticus* was exposed to a sublethal of selecron, it revealed a significant decrease in the RBCS counts, Hb and MCH, MCHC (hypochromic anemia).This could be attributed to hemolysis, is preceded by erythrocyte swelling which leading to an increase of MCVand PCV. Selecron formulation caused a significant increase in the values of PCV and MCV in *O. niloticus* associated with macrocytic anemia. The macrocytic anemia may be due to the swelling of erythrocytes as a result of releasing of catecholamine hormones, (stress hormones), which release a consequence of hypoxia in living organism (Soivio & Oikari, 1976 and Perry & Reed, 1992). After 7 days, of exposure to cord formulation (at 0.390 mg/l) a significant decrease in the RBCS count, Hb, PCV and MCHC values was observed, this associated with hypochromic anemia. This could be due to early iron deficiency (Jain, 1993). Exposure of *O. niloticus* to selecron or cord formulations caused leukocytosis. Leukocytosis is a defense mechanism against exposure to the tested insecticide in *O. niloticus* In contrast, SanthaKumar *et al.* (1999) mentioned that no significant effects on the RBCS count,Hb and PCV were observed in *Heteropneustes fossilis* following exposure to monocrotophos at 14 days. Hussein *et al.* (1996) demonstrated a significant decrease in the RBC_S count,Hb and PCV as well as in the erythrocyte indices of *O. niloticus* exposed to fenvalerate and atrazine pesticides was recorded. The liver tissue posse's enzymatic machinery to carry out the energy production and detoxification in living organisms (Kulkarni and Mehrotra, 1973). Exposure of *O. niloticus* to selecron caused a significant increase in the activity of ALT at both concentrations of tested insecticide by 14 day and within the recovery period. Our data suggest the existence of heavy drain of metabolites during profenofos stress, since stress is known to induce elevation of aminotransferases (Kulkarni and Mehrotra, 1973). Our results agree with Sarbadhikary and Sur (1990), and Begum and Vijaraghavan (1995) have reported that stress induced elevation in the activity of aminotransferases (ALT & AST) in serum of *O. niloticus* following

Table (6): Effect of different concentrations of selecron and cord (profenofos) on lipid peroxidation in *O.niloticus*

Time / Parameter	14 days					Recovery Period (7 days)				
		Selecron Treatments		Cord treatments			Selecron treatment		Cord treatments	
	Control	0.566 mg/L	0.850 mg/L	0.234 mg/L	0.390 mg/L	Control	0.566 mg/L	0.850 mg/L	0.234 mg/L	0.390 mg/L
Malondialdehyde (M.DA) nmol / gm tissue	201.04 ± 8.790	256.383 ± 10.216***	209.910 ± 24.292	159.643 ± 10.941*	171.563 ± 10.695	175.889 ± 10.559	232.127 ± 12.133**	181.189 ± 8.219	143.165 ± 13.030	190.875 ± 13.438

Values shown are mean ± S.E.

* P < 0.05 ** P < 0.01 *** P< 0.01

exposure to methyl-parathion as well as in serum of *Claries batrachus* after treatment with dimethoate. However, other investigators have observed no effect on aminotransferases (ALT & AST) activity in carp post-exposure to methidothion or paraquat (Vig *et al.*, 1987). The hepatotoxicity may be attributed to the depletion of glutathione in hepatocytes and inability of treated fish repletion of glutathione this in turn, caused the necrosis of liver tissues. It has been reported that depletion of glutathione occurred as a result of releasing the corticosteroids (stress hormone), which play role in deficiency of glutathione in hepatocytes (Vina, 1992). As result of exposed fish *O. niloticus* to selecron at higher concentration, the urea level was elevated markedly over the experimental period. Decrease in the rate of excretion of urea nitrogen, this produce an increase in the concentration of BUN in plasma (Coles, 1986). A remarkable elevation in levels of creatinine in *O. niloticus* following exposure to selecron and cord formulations was noted throughout the experimental period. The measurement of serum creatinine may provide a crude index of glomerular filtration. The decrease of glomerular filtration rate (G.F.R.), will result in an increase in concentration of serum creatinine, (Parums, 1996). Hussein *et al.* (1996) have reported that when *O. niloticus* was exposed to sublethal concentration of atrazine for 14 and 28 days, the urea level, was significantly unaffected throughout the experimental period. In contrast, El-Said (1997) showed that *Clarias lazera* when exposed to fluridane for 60 days, the urea level was elevated markedly, whereas the creatinine level did not affect. Lipid peroxidation is an oxidative deteriorative process of unsaturated fatty acids due to excess generation of free radicals that causes in injury of cells. The level of malondialdehyde (MDA) as indicator of free radical induced lipid peroxidation (Reed, 1994). Our results showed that elevation markedly of MDA levels in hepatocytes of *O. niloticus* at lower concentration of selecron after 14 days of exposure and during the recovery period. This may caused by reducing GSH content, and/or may be due to inhibition of the cytochrome P450 in hepatocytes of *O. niloticus*, consequently, the lipid peroxidation was elevated markedly. In contrast, exposure of *O. niloticus* to cord formulation at lower concentration, exhibited a marked decrease in MDA levels, compared with control fish, this may be due to induce the cytochrome P450, mediated by pipronyl butoxide, which caused an increase in the metabolism and elimination

rate of such insecticide, thus, lessened the cytotoxicity of hepatocytes in *O. niloticus* (Hodgson and Levi, 1998).Our results agree with Ours and Uner (2000) and Uner *et al.* (2001). They have reported that the lipid peroxidation was markedly increased in fresh water *O. niloticus* and *Carpio carpio* following exposure to azinophos-methyl and cypermethrin pesticide, respectively.

IR:

The spectrum of profenofos has not been examined by IR. It shows no difference between cord and selecron formulations Fig. (4).

GC/MS analysis:

The GC/MS analysis of selecron and cord formulations indicated that existence biperonyl butoxide in the cord formulation; on contrast the biperonyl butoxide is not found in the selecron formulation Figures (5 & 6).

3) Residues analysis of selecron and cord formulations in *O. niloticus* tissues:

The results in Table, 7 exhibit that the residues analysis of selecron formulation insecticide at both concentrations in different organs and muscle flesh of fish. After 7 days, the residues levels at low concentration (0.566 mg/l) were 5.85, 1.68, ND, 0.37, 0.15, 0.19, and 0.17 mg/10g wet tissues respectively and were 7.86, 2.44, 0.27, 0.62, 0.45, 1.72, and 0.57 mg/10 g wet tissues after 14 days of application while the residues after recovery period for 7 days were 2.74, 0.54, ND, 0.25, 0.20, 0.96, and 0.15 mg/10 g. wet tissues, But the residues of selecron formulation at high concentration were 9.25, 0.38, 0.34, 0.61, 0.22, 0.37, and 0.42 mg/10 g wet tissues and were 8.15, 3.10, 0.61, 1.66, 0.52, 1.62, and 0.85 mg/10 g after 14 days, while the residues of selecron after recovery period for 7 days were0.31, 0.16, 0.002, 0.09, 0.057, and 0.0002 mg/10 g wet tissues in liver, kidney, brain, spleen, heart, gills, and muscle flesh, respectively. The results in Table, 8 exhibit that the residues analysis of cord formulation at low concentration (0.234 mg/l) were 2.74, 0.54, ND, 0.25, 0.20, 0.96, and 0.15 mg/10 g wet tissues and after 14 days were 3.23, 1.48, 0.08, 0.27, 0.15, and 0.211 mg/10 g wet tissues, days. But the residues of cord formulation at high concentration (0.390 mg/l) were 3.38, 1.85, 0.24, 0.71, 0.32, 1.12, and 0.26 mg/10 g wet tissues after 7 days and were 4.13, 2.02, 0.44, 0.74, 0.37, 1.45, and 0.36 mg/10 g wet tissues after 14 days of application, while the residues levels after recovery period for 7 days were 0.15, 0.03, ND, ND, ND,

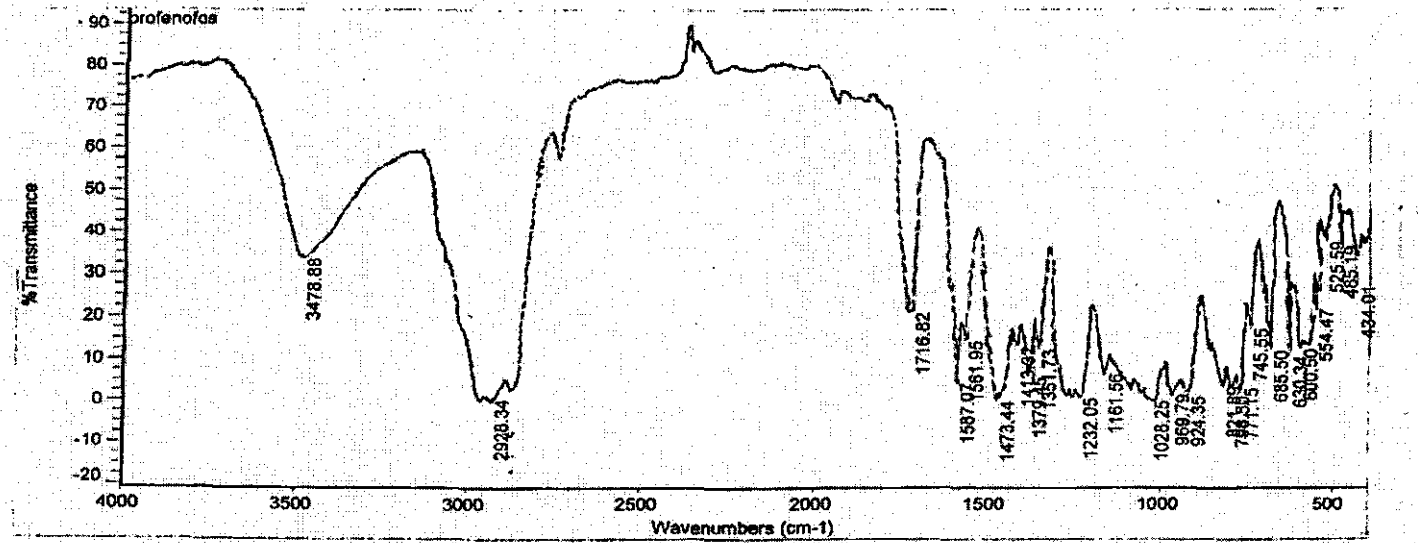

Figure (4): Infrared spectrum of the profenofos in cord and selection formulation

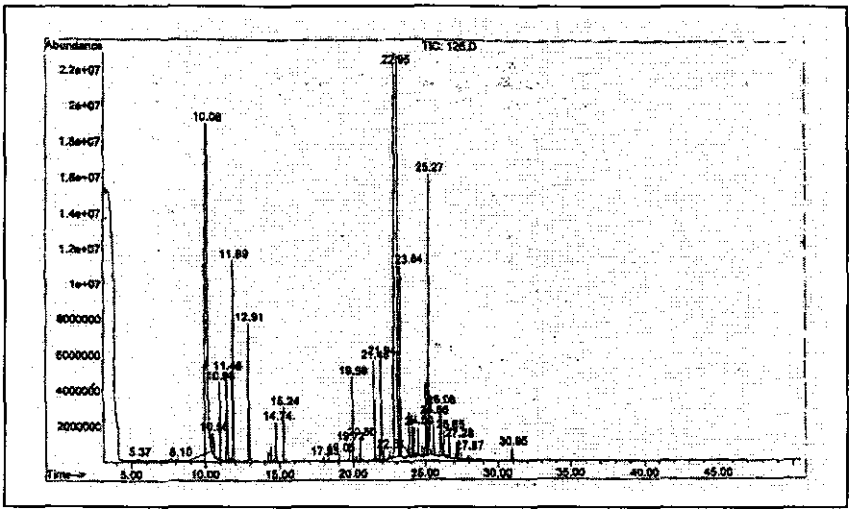

Figure (5): Expanded section of (RIC) for cord formulation.

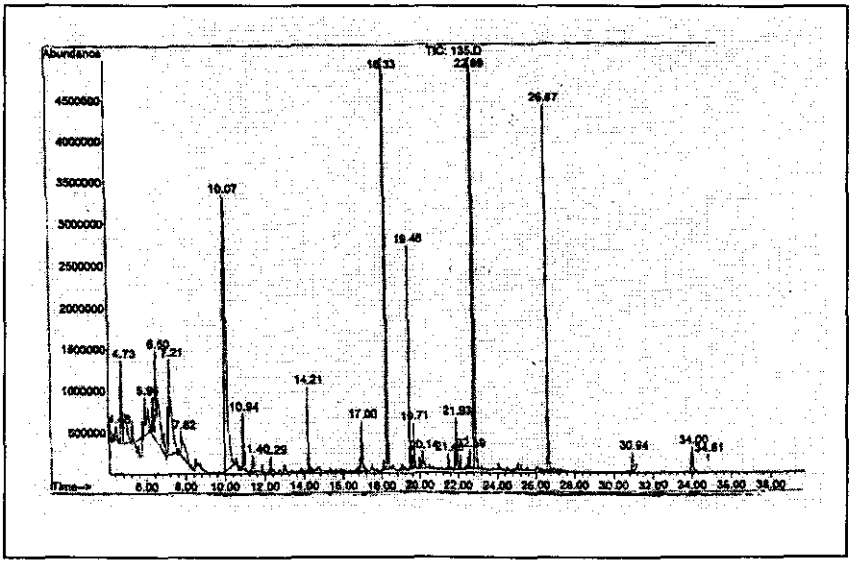

Figure (6): Expanded section of (RIC) for selecron formulation.

Table (7): Residues analysis of different concentrations of selecron (profenofos) in different organs and flesh of fish after 7 and 14 days of exposure and during the recovery period.

Insecticide / Tissues	7 days				14 days				Recovery for 7 days			
	0.566 mg/L		0.850 mg/L		0566 mg/L		0.850 mg/L		0566 mg/L		0.850 mg/L	
	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g we tissue	%
Liver	5.852 ± 0.043	25.85	9.254 ± 0.1212	27.22	7.862 ± 0.006	34.73	8.155 ± 0.060	23.99	0.246 ± 0.0423	1.086	0.318 ± 0.01	0.0935
Kidney	1.683 ± 0.012	7.43	0.387 ± 0.0005	1.14	2.444 ± 0.0215	10.79	3.105 ± 0.0129	9.13	0.182 ± 0.017	0.803	0.168 ± 0.016	0.492
Brain	ND	-	0.3432 ± 0.003	1.01	0.274 ± 0.0004	1.21	0.619 ± 0.0026	1.82	ND	ND	0.002 ± 0.0001	0.001
Spleen	0.375 ± 0.0001	1.66	0.6161 ± 0.005	1.81	0.628 ± 0.0045	2.77	1.065 ± 0.0079	3.13	0.036 ± 0.0031	0.157	0.090 ± 0.0083	0.264
Heart	0.152 ± 0.001	0.67	0.2274 ± 0.0017	0.67	0.458 ± 0.0006	2.02	0.5218 ± 0.005	1.54	ND	ND	ND	ND
Gills	0.190 ± 0.0003	0.76	0.3739 ± 0.0027	1.09	1.723 ± 0.0071	7.61	1.6200 ± 0.0119	4.77	0.036 ± 0.0016	0.0158	0.057 ± 0.005	0.169
Muscle	0.171 ± 0.0001	0.84	0.4210 ± 0.0003	1.24	0.571 ± 0.0042	2.52	0.8510 ± 0.0031	2.50	0.0002 ± 0.003	0.0007	0.0002 ± 0.002	0.0006

All Values = mean ± S.E.

ND = non – detecte

Table (8): Residues analysis of different concentrations of cord (profenofos) in different organs and flesh of fish after 7 and 14 days of exposure

Insecticide / Tissues	7 days				14 days				Recovery for 7 days			
	0.234 mg/L		0.390 mg/L		0.234 mg/L		0.390 mg/L		0.234 mg/L		0.390 mg/L	
	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%	mg/10g wet tissue	%
Liver	2.7499 ± 0.0119	29.38	3.383 ± 0.0239	20.72	3.2320 ± 0.0239	36.14	4.133 ± 0.0308	26.49	0.156 ± 0.018	1.670	0.150 ± 0.025	0.960
Kidney	05457 ± 0.0301	5.83	1.855 ± 0.008	9.51	1.484 ± 0.0131	19.82	2.0210 ± 0.0029	12.96	0.035 ± 0.003	0.369	0.035 ± 0.0033	0.224
Brain	No	ND	0.2483 ± 0.0018	0.55	0.086 ± 0.0008	2.65	0.446 ± 0.0019	2.86	ND	ND	0.002 ± 0.00002	0.012
Spleen	0.2558 ± 0.0019	2.73	0.713 ± 0.0063	1.77	0.2762 ± 0.0042	7.62	0.7419 ± 0.0043	4.76	ND	ND	0.016 ± 0.002	0.010
Heart	0.204 ± 0.0015	2.18	0.3209 ± 0.0014	1.01	0.158 ± 0.0007	3.44	0.378 ± 0.003	2.42	ND	ND	ND	ND
Gills	0.966 ± 0.0072	10.32	1.124 ± 0.0083	7.47	1.166 ± 0.0103	12.01	1.456 ± 0.005	9.33	0.006 ± 0.002	0.062	0.007 ± 0.002	0.043
Muscle	0.1510 ± 0.0011	1.61	0.264 ± 0.0023	1.35	0.211 ± 0.001	2.82	0.3671 ± 0.0011	2.35	0.001 ± 0.0016	0.011	0.001 ± 0.0002	0.004

All Values = mean ± S.E.
ND = non – detected

0.006, 0.001 mg/10 g wet tissues. The high uptake and penetration within tissues of organ phosphorus insecticides via integument of Tilapia fish was also observed by (El-Sheamy *et al.*, 1991).

4) Persistence of selecron and cord formulation in water :

Data in Table, 9 indicate that profenofos at cord formulation showed high disappearance in aquaria water compared with selecron formulation. The time required to disappearance of 50% of the added concentration ($T_{1/2}$'s) of selecron insecticide, reached to 167.24 and 166.65 h. In selecron formulation, and 113.33 and 53.11 h.in cord formulation at low and high concentrations respectively, then decreased to 4.04 and 5.53 mg/100 ml.water in selecron formulation. While the cord formulation concentration decreased to 1.60 and 2.34 mg/100 ml water after 14 days of treatment at low and high concentrations respectively. The rapid disappearance of cord and selecron formulations in aquaria water may resulting from chemical degradation as well as volatilization and fish absorption during experimental period. The penetrable of cord formulation was more than selecron formulation through fish skin and gills. The compounds were added during the industrialization process such as impurities (4-bromo-2-chlorophenyl) which produced from profenofos degradation. The permissible concentration of 4-bromo-2-chlorophenyl in profenofos formulations (selecron and cord) was less than 1% (FAO specification 2002). The HPLC analysis of- 4-bromo 2-chlorophenyl indicated that the levels of this impurity within the permeable level but it's concentration in selecron higher than cord formulation.

The GC/MS analysis of selecron and cord formulations indicated that existence biperonyl butoxide in the cord formulation as a synergist. This synergist which caused the high penetration and higher toxicity effects compared with selecron formulation. Penetrability lead to higher residue levels of insecticide in fish treated with cord formulation than in fish treated with selecron formulation. The low persistence of other organ phosphorus pesticides was also noticed by Beynon *et al.* (1971), Moody *et al.* (1987) and Afifi *et al.* (2002).

Table (9): Persistence rate of different concentrations of cord and selecron (profenofos) in water at different periods

Insecticide	Selecron				Cord			
Conc. / Time hr.	0.566 mg/L		0.850 mg/L		0.234 mg/L		0.390 mg/L	
	mg/100ml	%	mg/100ml	%	mg/100ml	%	mg/100ml	%
Zero	54.713 ± 0.858	96.666	83.791 ± 0.220	98.578	22.299 ± 0.1470	95.295	34.446 ± 0.4571	88.323
24 hr.	49.536 ± 0.408	87.519	79.263 ± 0.046	93.250	18.672 ± 0.01455	79.797	27.289 ± 0.4966	69.908
48	47.601 ± 0.703	84.010	77.192 ± 0.169	89.758	17.684 ± 0.3901	75.573	21.577 ± 0.3931	55.325
72	45.197 ± 0.116	79.854	68.31± 0.361	80.037	15.141 ± 0.566	64.707	18.236 ± 0.0001	46.758
96	44.938 ± 0.176	79.396	62.369± 0.419	73.375	13.8123 ± 0.1474	59.028	16.941 ± 0.135	43.439
120	39.441 ± 0.306	69.684	59.023 ± 1.140	69.439	10.619 ± 0.0005	45.384	14.181 ± 0.113	36.361
6d.	22.413 ± 0.203	39.599	55.231 ± 0.635	64.978	9.0678 ± 0.123	38.751	12.786 ± 0.579	32.785
7 d.	10.381 ± 0.001	18.345	31.822 ± 1.848	37.438	7.4578 ± 0.010	31.870	8.866 ± 0.1627	22.734
14 d.	4.0423 ± 0.032	7.141	5.539± 0.134	6.517	1.608± 0.0123	6.872	2.349 ± 0.018	6.022

All values shown are means ± S.E.

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دراسات كيميائية حيوية على متبقيات إثنين من مستحضرات مبيد البروفينفوس الحشري على أسماك البلطى النيلى

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أجريت هذه الدراسة للتعرف علي التأثيرات الضارة للتركيزات تحت المميتة للمستحضرين التجاريين لمركب البروفنيفوس وهي(٠,٥٦٦و٠,٨٥٠ مليجرام/لتر فى حالة السيليكرون و٠,٢٣٤ و٠,٣٩٠ مليجرام/لتر فى حالة الكورد) وذلك على صورة الدم علي صور الدم ووظائف الكبد والكلى وكذلك أكسدة الليبيدات في خلايا الكبد لأسماك البلطي النيلي حيث أظهرت النتائج نقص في عدد كريات الدم الحمراء وكذلك محتواها من الهيموجلوبين ومتوسط تركيز الهيموجلوبين في كرات الدم الحمراء (MCHC) وذلك في حالة التركيز المنخفض(٠,٥٦٦ مليجرام/لتر) لمستحضر السيليكرون بينما حدثت زيادة معنوية في حجم الكريات المنضغطة (PCV) ومتوسط حجم كريات الدم الحمراء (MCV) وكذلك متوسط تركيز الهيموجلوبين (MCV) وذلك في معاملات السيليكرون بعد ٧ أيام من المعاملة.وهناك ايضا نقص معنوي في عدد كريات الدم الحمراء و الهيموجلوبين وكذلك متوسط تركيز الهيموجلوبين في كريات الدم الحمراء في حالة التركيز العالي (٠,٨٥٠ مليجرام/لتر) من السليكرون بينما هناك زيادة معنوية في حجم الكريات المنضغطة ومتوسط حجم الكريات وكذلك متوسط تركيز الهيموجلوبين بعد١٤ يوم من المعاملة. لوحظ أيضا هذا الإتجاه في حالة العدد الكلي لخلايا الدم البيضاء في الأسماك المعاملة بالسيليكرون بعد ١٤ يوم من المعاملة. وقد لوحظ عدم عودة قيم تلك المعايير إلي مستوى المجموعة غير المعاملة (الكونترول) أثناء فترة الإستشفاء .في حالة المعاملة بالكورد لوحظ نقص معنوى فى عدد كريات الدم الحمراء فى حالة التركيز العالى(٠,٣٩٠ مليجرام/لتر)من الكورد بعد ٧ أيام من المعاملة وبينما كانت هناك زيادة معنوية في فى العدد الكلى لخلايا الدم البيضاء خلال فترة التجربة (١٤ يوم) وقد عادت القيم إلى معدلها الطبيعى أثناء فترة الإستشفاء. حدثت أيضا زيادة معنوية فى نشاط إنزيمات النقل الأمينى فى حالة المعاملة بالسيليكرون بعد ١٤ يوم من المعاملة وقد استمرت تلك الزيادة أثناء فترة الإستشفاء. فى حالة المعاملة بمستحضر الكورد حدثت زيادة معنوية فى انزيم الأسبرتيت أمينوترانسفيريزفي كلا المعاملتين بعد ٧ أيام وفي حالة التركيز المنخفض بعد ١٤ يوم من المعاملة وقد استمرت هذه الزيادة أثناء فترة الاستشفاء وكذلك حدثت زيادة معنوية فى مستوى اليوريا فى حالة التركيز العالى لكلا المستحضرين بعد ٧ أيام من المعاملة وكذلك فى حالة التركيز العالى من السيليكرون بعد ١٤ يوم من المعاملة وأيضا حدثت زيادة معنوية فى مستوى الكرياتينين فى معاملات السيليكرون أما فى حالة الكورد فقد حدثت الزيادة فى الكرياتينين فى حالة التركيز العالى بعد ٧ أيام من المعاملة وفي كلا المعاملتين خلال باقى فترة التجربة وقد حدثت أيضا زيادة فى مستوى أكسدة الليبيدات فى خلايا الكبد فى حالة التركيز المنخفض من السليكرون بعد ١٤ يوم من

المعاملة وأثناء فترة الإستشفاء بينما حدث نقص معنوى فى هذا المعيار فى حالة التركيز المنخفض(٠,٢٣٤ مليجرام/لتر) من مستحضر الكورد بعد ١٤ يوم وقد عادت القيم إلى معدلها الطبيعى أثناء فترة الإستشفاء. كما وجد أن أعلى مستوى من المتبقي للمستحضرات تحت الدراسة تم الكشف عنها فى كبد أسماك البلطى النيلى وعلى العكس أقل مستوى متبقى من كلا المستحضران (السيليكرون والكورد) تم الكشف عنه فى مخ الأسماك مع كل المعاملات بالإضافة لذلك وجد أن أعلى مستوى ثبات لكل من السيليكرون والكورد فى الماء تم الكشف عنه بعد المعاملة مباشرة وبعد ٢٤ ساعة من المعاملة. بينما زادت النسبة المئوية للفقد بمرور الوقت فى كلا المستحضرين لكل التركيزات. كما لوحظ أن معدل ثبات مستحضر الكورد كان أقل من السليكرون فى الماء بعد ١٤ يوم من المعاملة على التركيزات العالية و المنخفضة المستخدمة .أيضا تم الكشف والتعرف على المكونات المختلفة الداخلة فى كل المستحضران المستخدمان بإستخدام جهاز التحليل الكروماتوجرافى الغازى المتصل بجهاز مطياف الكتلة.

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