American-Eurasian Journal of Toxicological Sciences 11 (2): 29-38, 2019
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2019.29.38

Maternal and Cytotoxic Effects of Chlorfenapyr as a Pro-Insecticide in Pregnant Female Albino Rats

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Abstract: Chlorfenapyr (CFP), one of new chemical classes called pyrroles, used extensively in Egypt especially in the greenhouse. To better understand the maternal toxicity of CFP in female albino rats, 24 rats were orally administered at different doses (1/10 and 1/20 LD_{50} of CFP equivalent to 108 mg/kg Bw and 54 mg/kg Bw, respectively) daily from day 6th to day 15th of pregnancy with daily observation and weighing, the pregnant dams were sacrificed on day 20th of gestation. The hematological, biochemical, histopathological, ultrastructural changes examination and CFP residues in the liver tissues were used to evaluate the toxicity of the CFP in experimental animals. CFP oral administration, enhanced maternal toxicity evidenced by significant decrease in the female body weight and significant decrease in the metabolic parameters as glucose, total protein and cholesterol. However there was a significant increase in the liver enzymes, urea and creatinine furthermore there was a sever decrease in the antioxidant biomarkers as SOD, CT, GSH and GST with significant increase in the level of MDA revealing oxidative stress. Additionally the results showed that there was a sever histopathological and ultrastructural changes in the liver tissue and hepatocytes that was considered the main preferable organ for CFP toxicity besides other tissues as kidney, brain and spleen furthermore the level of CFP in the liver tissue increased in treated dams in comparison to control. Taken collectively, CFP could be a maternal toxic agent in female albino rats.

Key words: Chlorfenapyr · Greenhouse Insecticide · Maternal Toxicity · Chlorfenapyr residues · Ultrastructural Changes · Female Albino Rats

INTRODUCTION

The pesticides improper and extensive uses caused an inevitable pollution to soil, air, water and food with a detrimental hazard to non-target organisms and populations [1].

Pesticides occupational exposure during processing and application as workers and farmers are amongst the highest risk groups also thus exposed to lower concentration from contaminated food and water exert different and multiple biochemical alterations and ill health on the long term [2]. Chlorfenapyr, a pro-insecticide, has both a wide spectrum insecticide and acaricidal activity and act through inhibition of mitochondrial ATP production via uncoupling of oxidative phosphorylation disrupting the mitochondrial membranes proton gradient and prevent conversion of ADP to ATP with generation of free oxygen radicals and oxidative stress besides cellular death and organism mortality [3].

The highest concentration of radioactive absorbed chlorfenapyr is present in fat and liver and lowest concentration in brain besides sex differences, whereas concentrations of radioactivity in female higher than that of males by 2 or 3 folds and in general the main route of elimination is via faeces [4].

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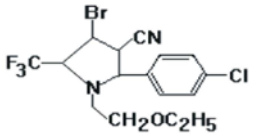
In a short term toxicity study in rats for 28 days with different concentrations of chlorfenapyr showed a dose dependent significant decrease in body weight and food consumption to a relative increase in livers and kidney weight with hepatocellular hypertrophy besides a significant increase in ALT, GGT, total albumin and BUN but there was no hematological changes recorded except an increase in leukocyte count and decrease in hemoglobin content [5]. Additionally, mice administered chlorfenapyr once orally at different doses (4.9, 9.8 and 19.6 mg/kg) that induced significant damage of kidney, liver and splenic cells and effect was greater in the renal and hepatic cells than splenic cells [6]. Chlorfenapyr different concentrations (250, 300 and 350 μM) caused oxidative stress induction and growth inhibition in the Paramecium sp. freshwater protozoa with a decrease in the glutathione (GSH) level and significant increase in glutathione S-transferase (GST) activity due to oxidative stress [7]. Notably, the cytotoxicity Studies of CFP are so limited, but the CFP cause induction and increase the expression of (HSP) heat shock protein genes in cabbage armyworm cell line that considered as a biomarker of oxidative stress to protect the living cells from oxygen free radicals that generated due to uncoupling of the mitochondrial oxidative phosphorylation upon exposure to CFP causing a sever cellular oxidative stress [8]. Also, chlorfenapyr acts through uncoupling oxidative phosphorylation and inhibit ATP production with cellular degeneration and death [9, 10].

The rationale of this study to evaluate the maternal toxicity of the CFP in female albino rats.

MATERIALS AND METHODS

Fungicide Chlorfenap: Chlorfenapyr is white to light brown suspension (SC) with characteristic sweetish odor and dispersible in water kindly obtained from the Central Agricultural Pesticide Laboratory, Doki, Giza, Giza Governorate.



Laboratory Animal: 24 Mature female albino rats obtained from the Experimental Unit in the Faculty of Pharmacy, Mansoura University; Animals weighed about 250 ±10 gm and were obviously healthy, then grouped and housed in plastic cages with soft wood shavings as a bedding material that changed adequately to ensures a low level of ammonia and to keep animals clean and dry.

Animals adapted for about 2 weeks and maintained on a balanced ration before the experiment also standard laboratory pelleted diet and water given ad libitium throughout the experiment and Light cycles of 12 hours' light to12 hours dark seemed to be adequate in order to promote rodents' breeding.

Calculation of LD50 of Cu Oxychloride: The LD50 of CFP was calculated according to the Up and Down Procedure (UDP) that proposed by Bruce [11] and revised and modified by Organisation for Economic Co-operation and Development (OECD) [12] and accepted as a method for calculation of LD50 through AOT 425 statistical program and the estimated and calculated LD50 was recorded by us earlier 1078 mg/kg [13].

Experimental Design for Prenatal Maternal Toxicity Study of Chlorfenapyrin Pregnant Female Albino Rats: Twenty four (24) synchronized pregnant females were separated into three groups with eight for each whereas the duration of exposure designated to be from day 6th to day 15th of pregnancy daily orally by stomach tube, the first group received 0.5 ml distilled water and used as control, the second group administered 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw., the third group gavaged1/20 LD_{50} of CFP equivalent to 54 mg/kg Bw, the pregnant females were weighed and kept under observation daily until the day 20th of gestation (The day of sacrifice).

Prenatal Maternal Toxicity of Dams upon Exposure to Chlorfenapyr

Clinical Signs: The treated pregnant females observed daily throughout the experimental period for any abnormal behavior, findings or alteration.

Maternal Body Weight Gain: The initial body weight was determined and then throughout the experiment the body weight was calculated before each administration. The body weight gain % was determined according to the following formula [14].

$$Body\ weight\ \% = \frac{Final\ body\ wt - initial\ body\ weight}{initial\ body\ weigth} \times 100$$

Sample Collection: At day 20 of pregnancy, pregnant dams euthanized with thiopental Na (40 mg/Kg i.p).

For hematological examinations fresh blood sample collected from the heart with a sterile syringe and then collected in centrifuge tubes containing K3EDTA as anticoagulant.

Table A: The experimental design summery for prenatal maternal toxicity of cu oxychloride in pregnant female albino rats

Group	No. of Pregnant rats	Treatment	Oral dosage mg/kg B.wt	Duration of exposure during pregnancy	Sacrificing
I	8	D.W	0.5 ml/rat	6^{th} to 15^{th}	20^{th}
II	8	Chlorfenapyr	108	6^{th} to 15^{th}	20^{th}
III	8	Chlorfenapyr	54	6^{th} to 15^{th}	20^{th}

For biochemical analysis fresh blood collected in gel tube (Not containing anticoagulant) then left overnight in refrigerator followed by serum separation in centrifuge at 3000 rpm for 15 minutes then stored at - 20? in Eppendorf tubes.

For Oxidative stress determination, liver sample was removed and washed with saline solution then one gram of tissues was homogenized in falcon tube with 9 ml ice cold phosphate puffer (PBS) PH7.4 through homogenizer then centrifuged at 3000 rpm for about 15 minutes at 4?, the supernatant was separated, collected and stored at - 20? in Eppendorf tubes [15].

For histopathological examination liver, spleen, kidney, brain and placenta specimen were collected and kept in 10% neutral buffered formalin.

Hematological Examination: Blood sample analysis was carried out by Mindray BC-1800 hematological analyzer whereas hemoglobin (Hb), red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were evaluated besides total and differential white blood cells were also measured [16].

Biochemical Analysis

Metabolic, Kidney and Liver Function Biomarkers: The activity of GGT was estimated according to Burtis and Ashwood [17] ALT [18] Glucose [19] total protein [20] albumin [21] creatinine [22] urea [23] and cholesterol level [24] in sera of all treated female'rats compared to control one.

Antioxidant and Oxidative Stress Biochemical Analysis: Liver homogenates were analyzed for GSH, GST, SOD, CAT and MDA

Determination of Reduced glutathione (GSH) was estimated according to Beutler [25], Glutathione - s - transferase (GST) was estimated according to Habing *et al*. [26], Superoxide dismutase (SOD) was estimated according to Nishikimi *et al*. [27], Catalase (CAT) was estimated according to Goth [28] and malonedialdehyde (MDA) was estimated according to Satoh [29].

Histopathologic Examination: Specimens from liver, kidney, spleen and brain were fixed in 10% formalin and 5µ thickness sections of specimens prepared then stained with hematoxylin and eosin (H&E) and examined microscopically [30].

Transmission Electron Microscope Examination: Liver samples with 1x2 mm thickness were Fixed in 2.5 % glutaraldehyde solution immediately after animal dissection for 24 - 48 h. all steps described earlier [13, 31] and finally examined by JEM 100 CXII electron microscope and photographed by XR- 41digital camera.

Determination of Chlorfenapyr Residues in Dams Liver Tissue: Acetate buffered QuEChERS approach was used for the extraction of pesticide residues in in liver samples and followed by GC-ECD analysis [32].

Statistical Analysis: All numerical results analyzed statically for variance by one-way ANOVA and least standard difference LSD according to Snedecor and Cochran [33] by computerized SPSS program version 20.

RESULTS

Prenatal Maternal Toxicity upon Exposure to Chlorfenapyr Clinical Signs after Chlorfenapyr Exposure: Pregnant females showed a relative decrease in the feed consumption and the body weight throughout the study in comparison to control especially at the higher dose (1/10 LD_{50} of CFP).

Chlorfenapyr treated females also showed signs of irritability, circling, abnormal gait and severe diarrhea especially at the higher dose (1/10 of LD_{50}) signs illustrated at Fig. (1).

Maternal Body Weight Gain % upon Exposure to Chlorfenapyr in Pregnant Female Rats: The results showed a relative significant decrease in body weight gain in all treated groups throughout the study in respect to the control group especially groups of 1/10 LD_{50} of CFP and results illustrated by Table (1) and Fig. (2).

Fig. 1: Showing pregnant rats treated with 1/ 10 LD_{50} of CFP exhibited irritability, circling, abnormal gait and severe diarrhea

Table 1: Showing initial and final body weight mean and body weight gain % in pregnant female rats administered orally different doses of CFP (mean ± SE)

Group	Mean Initial body weight	Mean Final body weight	Body weight gain %
Control	154.5±2.18	227.88±2.49[a]	47.5
Group 1/10 LD50 of CFB	155.5±2.61	195.25±1.35[b]	25.56
Group 1/20 LD50 of CFB	155.63±1.87	204.0±2.06[c]	31.08

a, b, c significantly at ≤ 0.05

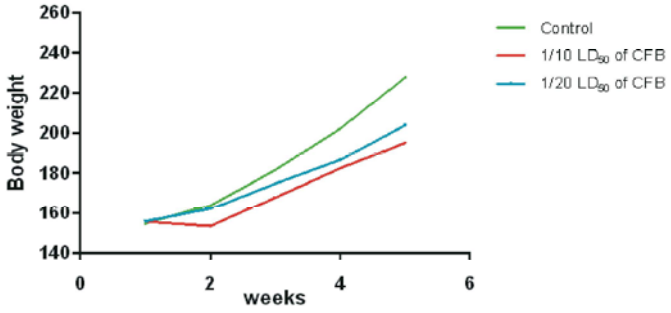


Fig. 2: Showing body weight mean per weeks after administration of different doses of CFP (1/10 LD_{50} and 1/20 LD_{50} of CFP equivalent to 108mg/kg Bw. and 54mg/kg Bw. respectively) orally from 6^{th} - 15^{th} days of pregnancy daily in comparison to the control group.

Prenatal Maternal Biochemical Analysis

Metabolic, Liver and Kidney Functions' Biomarkers: Estimation of Glucose, cholesterol, total protein, albumin, globulin, ALT, AST, GGT, urea and creatinine had been carried on after administration of different doses of CFP (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg Bw. and 54 mg/kg Bw. respectively) orally from 6^{th} - 15^{th} days of pregnancy daily in comparison to control group and results showed that:

For metabolic biochemical parameters there was a significant decrease of all metabolic biochemical parameters (Glucose, cholesterol, total protein, albumin and globulin) in all treated groups in comparison to the control group where there was a significant decrease in glucose, cholesterol, total protein, albumin and globulin in all treated groups in respect to the control group especially at dose level 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw.

For liver function biomarkers there was a significant increase of all biomarkers (ALT, AST and GGT) in all treated groups in comparison to the control group where there was a significant increase in ALT, AST and GGT in all treated groups in respect to the control group especially at dose level 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw.

For kidney function biomarkers there was a significant increase in blood urea nitrogen and creatinine in all treated groups in comparison to the control group especially at dose level 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw.

Oxidative Stress Biomarkers: Estimation of GSH, GST, SOD, CAT and MDA had been carried on after administration of different doses of CFP (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg Bw. and 54 mg/kg Bw. respectively) orally from 6^{th} - 15^{th} days

Table 2: Showed the biochemical metabolic, liver and kidney biomarkers changes after administration of different doses of CFPorally from 6th - 15th days of pregnancy daily in comparison to control group

	ALT (U/I)	AST (U/I)	GGT (U/I)	Glucose (mg/dl)	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	21.4±0.55^{c}	32.0±0.98^{c}	17.43±0.55^{c}	143.8±0.83^{a}	99.23±1.41^{a}	8.6±0.15^{a}	5.37±0.12^{a}	3.23±0.03^{a}	34.4±0.83^{c}	0.43±0.01^{c}
1/10 LD_{50} of CFB	48.14±0.90^{a}	63.07±1.43^{a}	44.33±0.61^{a}	79.43±0.58^{c}	52.47±0.52^{c}	4.5±0.15^{c}	2.72±0.11^{b}	1.93±0.2^{b}	72.66±2.19^{a}	1.86±0.03^{a}
1/20 LD_{50} of CFB	37.83±1.4^{b}	47.1±1.4^{b}	32.23±0.58^{b}	128.03±2.64^{b}	67.13±1.31^{b}	6.87±0.20^{b}	3.66±0.32^{c}	3.2±0.46^{a}	47.33±0.88^{b}	0.77±0.01^{b}

a, b, c significantly at ≤ 0.05

Table 3: Showed the biochemical oxidative stress biomarkers changes after administration of different doses of CFP orally from 6th - 15th days of pregnancy daily in comparison to control group

Groups	GSH mg/g. tissue	GST U/g. tissue	SOD U/g. tissue	CAT U/g. tissue	MDA nmol/g. tissue
Control	25.99±1.18^{a}	9.19±0.92^{a}	23.51±1.36^{a}	15.88±1.10^{a}	34.70±1.17^{c}
1/10 LD_{50} of CFB	15.33±0.95^{c}	5.56±0.50^{b}	14.24±1.78^{b}	10.89±1.71^{b}	57.06±2.04^{a}
1/20 LD_{50} of CFB	21.53±1.11b	6.79±0.64^{b}	18.75±1.79^{ab}	14.92±1.49^{a}	40.35±1.07^{b}

a, b, c significantly at ≤ 0.05

Table 4: Showed the hematological finding after administration of different doses of CFP orally from 6th - 15th days of pregnancy daily in comparison to control group

	RBCs (million cells/uL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Total WBCs (1000 cells/uL)
Control	8.12±0.05	14.55±0.05	45.78±0.07	56.37±0.46	17.92±0.17	31.79±0.12	7.13±0.05^{b}
1/10 LD 50 of CFP	7.98±0.07	13.99±0.26^{a}	44.94±0.39	56.30±0.71	17.52±0.20	31.13±0.55	8.61±0.07^{a}
1/ 20 LD 50 of CFP	8.14±0.09	14.29±0.06	45.47±0.29	55.87±0.61	17.55±0.18	31.42±0.07	7.41±0.09^{b}

a, b, c significantly at ≤ 0.05

of pregnancy daily in comparison to control group and results showed a significant decrease in GSH, GST, SOD and CAT in all treated groups when compared with the control groups especially the dose level 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw, On the other side MDA showed a significant increase in all treated groups in respect to the control group especially the dose level of 1/10 LD_{50} of CFP equivalent to 108 mg/kg Bw.

Hematological Finding: The results showed that there was a significant decrease in the Hb content count in the group treated with 1/10 LD50 of CFP equivalent to 108 mg/kg Bw., on the other hand total leukocytic count showed a significant increase in the groups treated with 1/10 LD50 of CFP equivalent to 108 mg/kg Bw., furthermore there was no significance change in PCV, MCV, MCH and MCHC levels in respect to the control values.

Histopathological Findings: The histopathological changes were observed after administration of different doses of CFP (108 mg/kg Bw. and 54 mg/kg Bw.) orally from 6th - 15th days of pregnancy daily in comparison to the control group and the results showed that there was a clear pathological changes especially at the higher dose level group.

Liver: Dams treated with different doses of CFP displayed lymphohistiocytic infiltration in hepatic parenchyma in a dose dependent manner, results illustrated in Fig. 3a.

Brain: Dams treated with different doses CFP showed showing showed neuronal necrosis and satellitosis besides hemorrhage replacing the brain parenchyma, results illustrated in Fig. 3b, c.

Kidney: Dams treated with different doses of CFP showeddissolution and congestion of the renal glomeruli in a dose dependent manner. in a dose dependent manner, results illustrated in Fig. 4 a, b.

Spleen: Dams treated with different doses of CFP showed lymphoid depletion with mild lymphocytic necrosis in white pulp in a dose dependent manner in a dose dependent manner, results illustrated in Fig. 4 c, d.

Transmission Electron Microscope Examination: The results showed that a clear morphological changes in the cellular structure and function in a dose dependent manner after administration of different doses of CFP (108 mg/kg Bw. and 54 mg/kg Bw.) orally from 6th - 15th days of pregnancy daily in comparison to the control group.

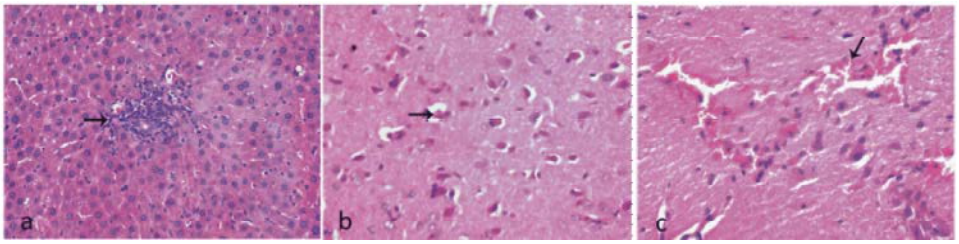


Fig. 3: Displayed (a) Liver of pregnant rat administered orally 1/10 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing lymphohistiocyticinfilteration in hepatic parenchyma (Arrow). (HE, 400x) (b) Brain of pregnant rat administered orally 1/10 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing neuronal necrosis and satellitosis (arrow). (HE, 400x), (c) Brain of pregnant rat administered orally 1/20 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing hemorrhage replacing its parenchyma (arrow). (HE, 400x).

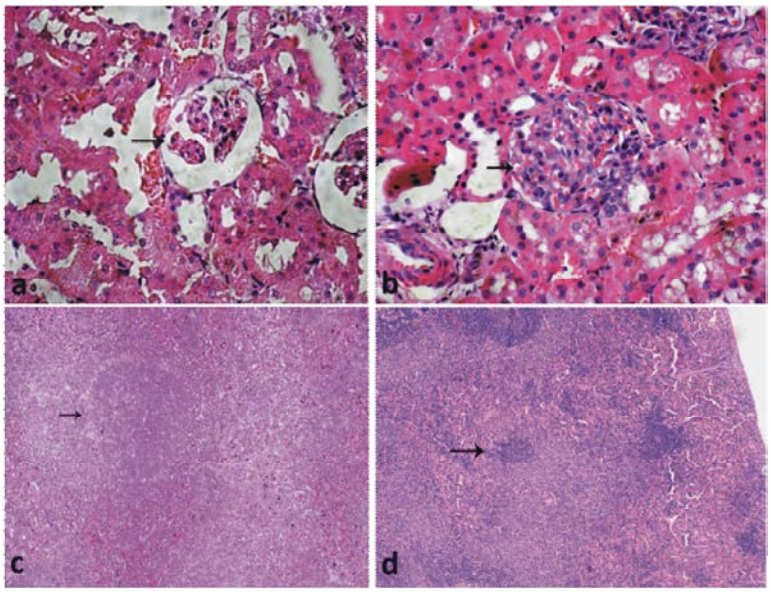


Fig. 4: Displayed (a) Kidney of pregnant rat administered orally 1/10 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing dissolution of the renal glomeruli (arrow). (HE, 400x), (b) Kidney of pregnant rat administered orally 1/20 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing is congestion of renal glomeruli (arrow). (HE, 400x), (c) Spleen of pregnant rat administered orally 1/10 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is sever showing lymphoid depletion (Arrow). (HE, 100x) and (d) Spleen of pregnant rat administered orally 1/20 of LD_{50} of CFB from 6th - 15th days of pregnancy daily is showing marked lymphocytic depletion (Arrow). (HE, 400x).

Treated dams with different doses of CFB showed mitochondrial and RER swelling in a dose dependent manner besides presence of large numbers of peroxisomes and homogenous electron lucent structureless materials under the nuclear membrane and around the nucleolus, results illustrated in Fig. (5).

Determination of Chlorfenapyr Residues in the Dams Liver Tissue

Linearity: A standard calibration curve of Chlorfenapyrat RT 2.534, the standard curve equation was Y = 1.52013X – 13020.62620 and correlation coefficient R2 = 0.99977, y is the peak area and x is the amount (ng/µl). The Linearity correlation is shown in Fig. 6.

Chlorfenapyr Residues in the Liver Tissue: The results showed that the level of CFP residues in the liver tissue increased after administration of 1/10 LD50 of CFP (Equivalent to 108 mg/kg Bw.) orally from 6th - 15th days of pregnancy daily in comparison to control group and the results showed in Table (5).

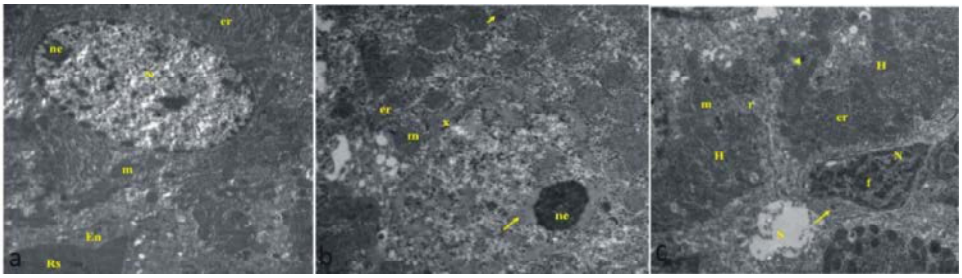


Fig. 5: Showing (a) T.E. micrograph of hepatic tissue displaying the hepatic cell having large vesicular nucleus (N) contain one or more nucleolus (ne) and the cytoplasm contain RER (er), mitochondria (m). Notice the hepatic sinusoid contain RBCs (Rs) and part from endothelial cell lining (En). (b) T.E. micrograph of hepatocytes of pregnant rat administered orally 1/10 of LD_{50} of CFB from 6th - 15th days of pregnancy daily displaying the nucleus (N) having electron dens nucleolus (Ne) surrounded by homogenous electron lucent area (Arrow), also disappearance of the nuclear membrane discontinuity and the chromatin clump appeared electron lucent (x), the cell organelles showing swelling such as mitochondria (m) and RER (er) besides increase in microbodies (Arrow head) and free ribosomes (r). (c) T.E. micrograph of hepatic cells of pregnant rat administered orally 1/20 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displaying hepatic sinusoid (S) with large number of kupffer cell having large nucleus (N) contain fat globule (f) and its cytoplasm contain cell organelles and lysosomes (Arrow). Notice the hepatic cells having cytoplasm contain swollen mitochondria (m), RER (Er), microbodies (Arrow head) and rich with free ribosomes (r).

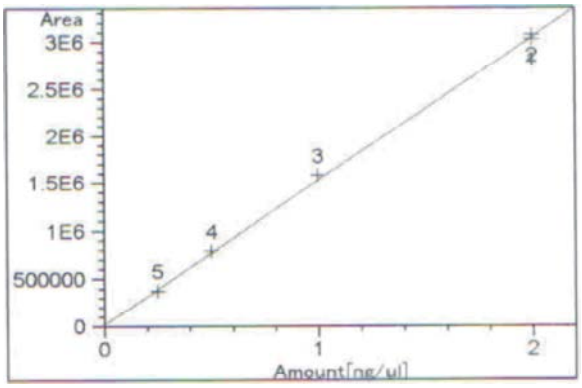


Fig. 6: Showed the Linearity correlation for Chlorfenapyr calibration curve

Table 5: Showed the level of CFP residues in the liver tissue of treated dams with different doses of CFP

Group	CFP level (ng/µl tissue)
Control	ND
1/10 of LD 50 CFP	0.0041
1/20 of LD 50CFP	ND

ND: non detectable

DISCUSSION

Toxic pesticides necessitate recognition of their hazards to the population health and ecosystem diversity whereas the toxicity mechanism of most pesticides occurs through free radical's production and antioxidants' alteration that play a crucial role in induction of oxidative stress and cellular oxidative damage [34].

For the Body weight, all treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study showed a significant relative decrease in the body weight in a dose dependent manner in respect to the control group and such results agreed with Metruccio and Boobis [5] and Saito [35] who confirmed that exposure to different concentration of chlorfenapyr 28 days in rats showed a dose dependent significant decrease in body weight and food consumption and might be explained according to Saito [35] and Black *et al.* [36] who stated that CFP depress the cellular growth and ATP production especially in highly active organs.

For Metabolic disorders' biomarkers, all treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study showed a significant decrease of all metabolic biochemical parameters as glucose, cholesterol,

total protein, albumin and globulin in a dose dependent manner in respect to the control group -liver considered as the most preferable organ for chlorfenapyr with bile retention and metabolic disruption so decrease its ability to absorb nutrients [37]. Similarly, a toxicity study investigated chlorfenapyr toxicity in male albino rats at dose of 0.45 mg/Kg b.wt. given daily for 28 days and found that chlorfenapyr caused a significant decrease total protein, globulin and albumin in all treated groups (38).

All treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study showed a significant increase in ALT, GGT, AST, Urea and Creatinine that reflect a state of hepatoxicity and nephrotoxicity after exposure to CFP in a dose dependent manner in respect to the control group and [5, 38, 39].

For oxidative stress biomarkers, all treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study showed a dose dependent decrease in GSH, GST, SOD and CAT and increase in MDA after exposure to CFP in a dose dependent manner in respect to the control group and such results agreed with Kegley *et al*. [40] who proposed that Chlorfenapyr insecticide act through interfering with the oxidative phosphorylation process in the mitochondria and deprivation of cellular energy and production of free radicals causing oxidative stress, also Benbouzid *et al*. [7] found that chlorfenapyr with different concentrations (250, 300 and 350 µM) caused oxidative stress induction and growth inhibition in the Paramecium sp freshwater protozoa with decrease in the glutathione (GSH) level due to oxidative stress. Moreover , Al-Sarar *et al*. [41] studied the cytotoxic effects of chlorfenapyr on the Chinese hamster ovary cells (CHO_{K1}) and the result showed a significant inhibition in the GST activity of treated cells and depletion of antioxidant cellular activity. Furthermore chlorfenapyr down regulated the serum heat shock proteins that used as a biomarker of the cellular stress that caused by environmental pollutants in a study carried by Zhang [42].

For hematological findings, all treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study showed a significant decrease in Hb content and on the other hand a significant increase in total leukocytic count after exposure to CFP in a dose dependent manner in respect to the control group and such results agreed with Jasper *et al*. [43] who confirmed that CFP induce a significant increase in the ROS production and RBCs membrane is very fragile and susceptible to oxidative stress that cause destruction and hemolysis of RBCs in the vascular system and on the other hand increase in the total leukocytic count related to the inflammatory response to the oxidative damage by CFP.

For histopathological and Ultrastructural findings, all treated dams with different doses of CFP (108 and 54 mg/kg Bw.) throughout the study a sever histopathological changes in the liver, kidney, brain and spleen also a significant morphological changes in the hepatocytes with ultrastructural degenerative changes after exposure to CFP in a dose dependent manner in respect to the control group and such results confirmed with the biochemical changes in the treated dams also according to Li and Chen [6] chlorfenapyr administration in mice induced a significant damage of kidney, liver and splenic cells and the effect was greater in the renal and hepatic cells besides Chlorfenapyr act through inhibition of mitochondrial ATP production via uncoupling of oxidative phosphorylation disrupting the mitochondrial membranes proton gradient and prevent conversion of ADP to ATP causing metabolic disruption with generation of free oxygen radicals and oxidative stress besides cellular death and organism mortality [3] so increase in the peroxisomes besides mitochondrial and RER swelling may be due to metabolic disruption with excessive production of ROS with successive degeneration of cellular organelles [44, 45].

For Chlorfenapyr residues level in the liver tissue, treated dams with 1/10 LD50 of CFP (108 mg/kg Bw.) showed increase in the level of CFP residues in the liver tissues in comparison to control and such results agreed with Albers *et al.* [37] who suggested that liver considered as the most preferable organ for chlorfenapyr with bile retention and metabolic disruption so decrease its ability to absorb nutrients in a toxicity study in male mallard ducks with different concentration of chlorfenapyr, also such results agreed with Eman and Basem [38] who found that chlorfenapyr may accumulate in different tissues in treated rats but such rats showed recovery after a period of time whereas CFP is rapidly metabolized and excreted in all mammals, fish and birds to environment. So Biological Control of pesticides by Microorganisms may be a tools for environmental protection [46].

CONCLUSIONS

On conclusions, CFP has cytotoxic effects on pregnant rats revealed by hematological, biochemical , pathological changes and alteration of antioxidant markers.

All authors have no conflict of interest.

ACKNOWLEDGMENTS

To Dr. Amr Elbawady for his help in obtaining and analysis of the insecticide from Central Agricultural Pesticide Laboratory, Ad Doki, Giza, Giza Governorate We also grateful thanks for prof dr ALLAM Nafady Assuit university, Egypt who help us in electron microscope scanning of fixed tissue.

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