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Developmental and Ultrastructure Toxicity of Greenhouse Insecticide Chlorfenapyr in Rat Fetuses

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Abstract: Pesticides are used extensively in the Middle East countries including Egypt to maintain several crop production under restricted precaution from agriculture ministry. To better understand the safety of Chlorenapyr, as an insecticide used in greenhouses and its developmental toxicity on female albino rats. Chlorenapyr (CFP) administered orally at different doses (1/10 and 1/20 calculated 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, the pregnant dams were sacrificed at day 20th of gestation and feti were weighed and their lengths were measured with assessing the external, visceral and skeletal malformations. The ultrastructural microscopical examination of the fetal liver was carried on. The results showed that there was a significant dose dependent decrease in the fetal weights and lengths in respect to the control group besides a representative increase in the external, visceral and skeletal malformations with increasing the dose. Microscopically, the fetal livers showed a significant increase in a dose dependent manner in the microbodies and peroxisomes in the hepatocytes besides swelling in the cytoplasmic organelles such as the mitochondria and the endopasmic reticulum(RER) with abnormal nuclear membrane. Such results revailed the potential teratogenicity of CFP insecticide in a dose dependent manner.

Key words: Chlorfenapyr · Visceral and skeletal malformations · Teratogenicity · Ultrastructure · Female Albino rats

INTRODUCTION

Agricultural chemicals recently proved that pesticides especially insecticides are indispensable in crop protection from weeds and pests for increasing the crop productivity [1]. However insecticides became less effective recently because of the development of resistance by some insects due to the heavy application of insecticides causing a catastrophic public and environmental impact [2]. Notably, workers and greenhouse farmers are amongst the highest risk groups exposed the pesticides during processing and application and this exposure to even lower concentration from contaminated food and water exert different and multiple biochemical alterations and ill health on the long term [3].

Chlorfenapyr, as a novel insecticide, is being used to control mites and caterpillar pests that are resistant to organophosphate, carbamate and pyrethroid insecticides specifically in vegetable and ornamental crops cultivated in greenhouses and not recommended for the surface agriculture and outdoor uses on cotton crops due to its chemical persistence in environment with a great hazardous concern on the food chain and animal production [4]. Chlorfenapyr, one of new chemical classes called pyrroles, is a pro insecticide that used mainly in greenhouse crops and vegetables and acts after metabolic activation with uncoupling of the mitochondrial oxidative phosphorylation and disrupts ATP production causing cellular death [5]. Chlorfenapyr is activated in vivo by oxidative removal of N-ethoxymethyl group through

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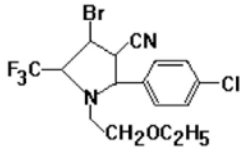
oxidation considered one of recent N-substituted halogenated pyrrole and has both a wide spectrum insecticide and is considered a perfect insecticide for eradicating malaria vectors [6, 7].

The distribution of absorbed chlorfenapyr after a radiolabeled test showed a highest concentration of radioactivity in fat and liver and lowest concentration in brain besides sex differences whereas concentration of radioactivity in female higher than that of male by 2 or 3 folds and in general the main route of elimination is via feces [8].

The rationale of this study aimed to investigate teratogenicity of chlorfenapyr in female albino rats and its effects on tissue ultrastructure of feti.

MATERIAL AND METHODS:

Insecticide: CHALLENGER SUPER 24% SC (chlorfenapyr) or (CFP): White to light brown Suspension Concentrate (SC) with characteristic sweetish odor and kindly obtained from Central Agricultural Pesticide Laboratory, Al Doki, Giza, Giza Governorate



Laboratory Animals: A thirty-nine mature pregnant female rats and 16 mature males albino rats were obtained from Experimental Unit in the Faculty of Pharmacy, Mansoura University. Animals had a mean body weigh of 250 ±10 gm and were 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 were adapted for 2 weeks and maintained on a balanced ration before the experiment in order to promote rodents' breeding.

Calculation of LD_{50} of Chlorfenapyr: The LD_{50} of CFP was calculated according to the Up and Down Procedure (UDP) that was proposed by Bruce [9] and revised and modified through OECD [10] and accepted as a method for calculation of LD_{50} through AOT 425 statistical program and the estimated LD_{50} was 1078 mg/kg. N.B. 15 female albino rat were used in this side experiment.

Determination of Zero Day of Pregnancy: Twenty-four Females were checked daily by vaginal smearing examination and only females in late proestrus or early estrus were mated with males through pairing of three females with one male. On the next each morning females checked by vaginal smear examination until presence of sperms or copulation plug and the date designated to be the zero day of pregnancy (GD 0) [11].

Experimental Design for Prenatal Toxicity Study of Chlorfenapyr on Developing Fetuses: Tested compound administered orally at different doses (1/10 and 1/20 calculated LD_{50} of CFP equivalent to 108 mg/kg BW and 54 mg/kg BW respectively) daily from day 6^{th} to day 15^{th} of pregnancy, the pregnant dams were sacrificed at day 20^{th} of gestation and feti were weighed and their lengths were measured with assessing the external, visceral and skeletal malformations (Table 1).

External Examination: After pregnant dams euthanized with thiopental Na, They were laparotomized for thoracic and abdominal cavities examination. Pregnancies confirmed by uterine examination, then the uteri were removed, opened with a scissor and uterine contents as resorption or implantation sites (corpora lutea numbers correspond to implants number), dead and live feti were recorded [12]. The feti then pulled out weighed and euthanized with hypothermia then carefully evaluated externally from head to tail for any abnormalities, the crown rump length and breadth of each fetus were recorded. Finally, half of feti injected with Bouin's solution intraperitoneally for visceral and histopathological examination and the other half was preserved in ethyl alcohol 95% for skeletal examinations [11, 13].

Table 1: The experimental design summery for prenatal developmental toxicity of chlorfenapyr.

Group	No. of Pregnant rats	Treatment	Oral dosage mg/kg B.wt	Interval 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}

Distilled water (D.W.)

Visceral Examination by Wilson's Technique: The soft tissue evaluation was performed according to Wilson's Technique that involves the fixation and decalcification of feti in Bouin's solution then the thoracic viscera as diaphragm and lungs were examined for herniation and abnormal lobulation respectively, the heart was examined carefully and sectioned transversally at the ventricles for the detection of any abnormal thickening in the ventricular wall then the abdominal viscera examined also for abnormal shape and fusion. The kidneys were examined also for their size and location and each kidney was cut transversely for any renal pelvis dilatation, craniofacial region serially cross sectioned (five sections) with a razor blade all sections examined by the dissecting microscope [12].

Skeletal Examination: After the feti were dehydrated in ethyl Alcohol 95% for about 2 weeks, feti were skinned and eviscerated through a ventral midline incision followed by staining either through single staining technique (for ossified tissue staining only) or double staining technique (for both ossified and cartilaginous tissues staining).

For the single staining technique, the eviscerated and skinned feti were placed in KOH 2% solution for about 24-36 h for soft tissue clearance then were stained with alizarin red (S) stain (0.1g alizarin red (S)) in the working stock solution (7 ml KOH 4% +60 ml Glycerin + DW up to 300 ml) for 24 h then feti were washed in working stock solution alone for another 24 h then put in glycerin different concentration (20, 50, 80, 100%) for 48h for each concentration and kept in pure glycerin 100% until evaluation and photography.

For the double staining technique eviscerated and skinned feti were placed in alcian blue stain solution (15 mg alcian blue + 80ml ethanol 95% + 20ml pure glacial acetic acid) for 24h then placed in ethanol 95% for another 24 h then stained with alizarin red (S) stain in KOH 2% stock solution (25mg of alizarin + liter of KOH 2%) for 24- 36 h then feti were removed and washed in the working stock solution for another 24 h then put in glycerin different concentration (20, 50, 80, 100%) for 48h for each concentration and kept in pure glycerin 100% until evaluation and photography [11].

Transmission Electron Microscope Examination: Liver samples with 1x2 mm thickness were taken and fixed in 5 % glutaraldehyde solution immediately after animal dissection for 24 - 48 h. Then specimens were washed in cacodylate buffer (PH 7.2) for 4 times and for 20 minutes each time followed by fixation in 1 % O_4S_4 for 2 hours then washed in the same buffer for four times again. Dehydration then applied in ascending manner with different alcohol concentrations (30-50- 0-90 and 100% for 2 hours in each concentration then embedded in epon- araldite mixture, the embedded blocks then cut by ultramicrotom in 0.5-1μ thickness and then ultrathin sections using Leica AG ultramicrotom made with 500-700 A thickness and then contrasted in lead citrate and uranyl acetate and examined by JEM 100 CXII electron microscope and photographed by XR- 41 digital camera [14].

Statistical Analysis: Data recorded in the current experimental teratogenic study were statistically analyzed for variance (ANOVA) and least significant difference (LSD) as described by Snedecor and Cochran (15) by using computerized SPSS program version 32.

RESULTS

External Examination Findings

Fetal Body Weight: Estimation of the mean fetal body weight showed that there was a significant decrease in maternally treated groups (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BWW. respectively) in dose dependent manner in respect to the control group (Table 2).

Fetal Crown-Rump Length: Estimation of the mean fetal crown-rump length showed that there was a significant decrease in in treated groups (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BW. respectively) in dose dependent manner in respect to the control group and results observed in table (2).

Embryonic Death (Resorption and Stillbirth) Rate: Fetal death (resorption) either partial or complete resorption and stillbirth (late embryonic death) were recorded and results indicated an increase in the rates of embryonic death maternally treated groups (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BW. respectively) in dose dependent manner in respect to the control group

The rate of partial resorption in pregnant females treated with 108 mg/kg BW. and 54 mg/kg BW. CFP was about 37.5% for both doses groups while complete resorption observed only in the dose group108 mg/kg BW. with rate of 12.5% of the total examined pregnant uteri.

Table 2: Fetal body weight and crown rump length of feti of treated dams with different doses of Chlorfenapyr orally from 6th - 15th days of pregnancy (Mean ± SE):

Group	Fetal body weight/g	Fetal crown-rump length/cm
Control	5.5^{a}±0.16	4.58^{a}±0.11
1/10 LD_{50} CFP	3.83^{c}±0.18	2.97^{c}±0.19
1/20 LD_{50} CFP	4.55^{b}±0.10	3.68^{b}±0.10

A, b, c: Different letters are significantly different between groups at P≤0.05

Table 3: showed rate of embryonic death, External and Visceral malformations of feti of treated dams with different doses of CFP

Group	Total number of pregnant rats	Total number of fetuses	Total number of examined fetuses	Embryonic death			External malformations of examined fetuses				Visceral malformations of examined fetuses					
				Resorption in pregnant dams		Still birth of fetuses	S\C hemorrhage	hydrocephalus	malformed limbs	opened eyes	cerebral hemorrhage	cleft palate	myocardial thickening	malformed kidneys	lung lubes fusion	misshaped livers
				Partial N (%)	complete											
Control	8	62	31	0	0	0	1 (3.2%)	0	0	0	0	0	0	0	0	0
1\10 LD50 of CFP	8	43	21	3 (37.5%)	1 (12.5%)	9 (20.9%)	8 (38.1%)	2 (9.5%)	6 (28.6%)	2 (9.5%)	6 (28.6%)	4 (19%)	5 (23.8%)	5 (23.8%)	3 (14.3%)	5 (23.8%)
1\20 LD50 of CFP	8	52	26	3 (37.5%)	0	4 (7.7%)	6 (23.1%)	0	2 (7.7%)	0	3 (11.5%)	1 (3.8%)	3 (11.5%)	2 (7.7%)	0	1 (3.8%)

The rate of still birth of maternally treated feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 20.9% and 7.7% of the total number of feti, respectively. The results are presented in Table (3).

External Malformations: Feti of the treated dams with (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BW. respectively) displayed several external anomalies and abnormal features in dose dependent manner in respect to the control group as subcutaneous hemorrhage or hematoma, hydrocephalus, internal hemorrhage and anomalies of the limbs and also high rate of external malformations observed in the higher dose

The rate of subcutaneous hemorrhage of control feti was about 3.2% of the total examined feti, while treated feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 38.1% and 23.1% of the total number of examined feti, respectively.

The rate of hydrocephalus of treated feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 9.5% and 0% of the total number of examined feti, respectively.

The rate of malformed limbs of maternally treated feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 28.6% and 7.7% of the total number of examined feti, respectively.

The rate of opened eyes malformation of treated feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 9.5% and 0% of the total number of examined feti, respectively. The results observed in Table (3) and Fig 1 (a, b, c)

Visceral Malformations: Feti of treated dams of dams treated with (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BW. respectively) exhibited different visceral malformations in a dose dependent manner when compared to the control group as cerebral hemorrhage, dilatation of the ventricles, cleft palate, thickening in the myocardium, abnormal shaped and positioned kidneys besides defect in shape and lobulation of their livers and lungs, respectively. The rate of cerebral hemorrhage of maternally feti with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 28.6% and 11.5% of the total number of examined feti, respectively.

The rate of cleft palate of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 19% and 3.8% of the total number of examined feti, respectively.

The rate of myocardial thickening of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 23.8% and 11.5% of the total number of examined feti, respectively.

The rate of malformed kidneys of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 23.8% and 7.7% of the total number of examined feti, respectively.

The rate of lung lubes fusion of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 14.3% and 0% of the total number of examined feti, respectively.

The rate of misshaped livers of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 23.8% and 3.8% of the total number of examined

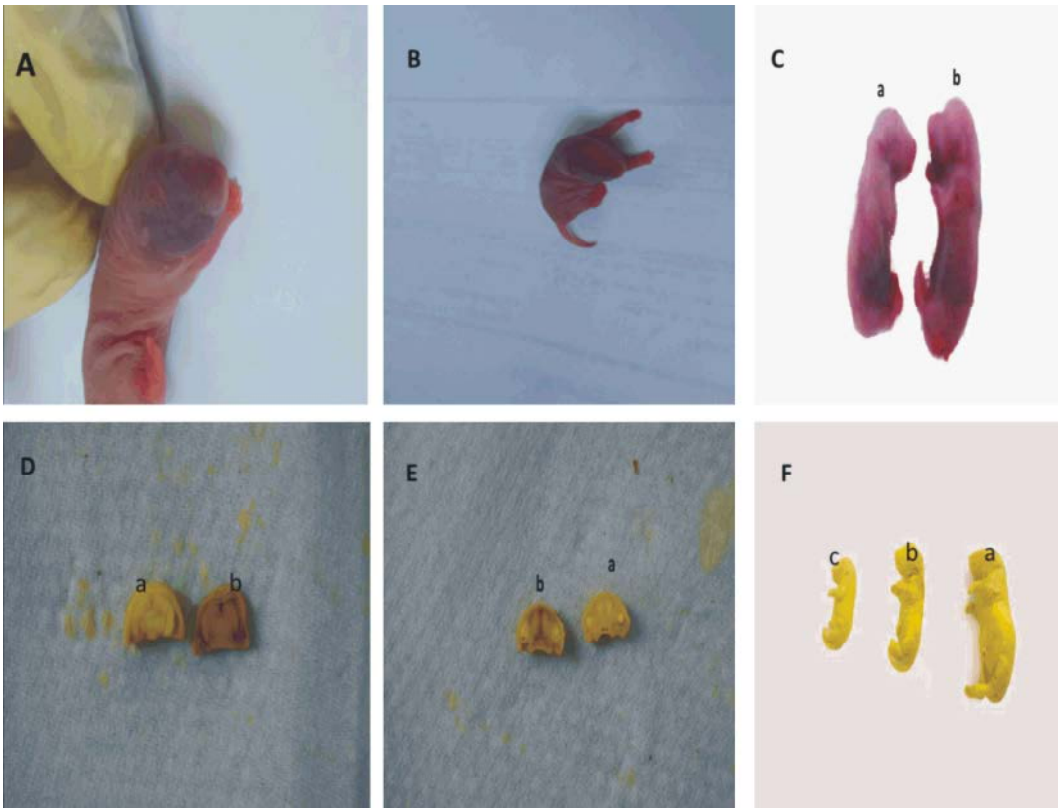


Fig. 1: a. maternally treated fetus with 1/10 LD_{50} CFP displayed with deformed twisted hind limbs. B. maternally treated fetus with 1/10 LD_{50} of CFP displayed sever cyanosis of the head with SC hemorrhage and ecchymosis. C. (a) maternally controlled fetus (b) maternally treated fetus with 1/10 LD_{50} of CFP displayed hydrocephalus. D. (a) normal olfactory bulb (cerebral hemisphere) of maternally controlled fetus (b) excessive hemorrhage in the olfactory bulb (cerebral hemisphere) of maternally treated fetus with 1/10 LD_{50} of CFP. E. a) normal cerebral hemispheres (right and left) and thalamus of maternally controlled fetus (b) hemorrhage and atrophy of the cerebral hemispheres (right and left) and thalamus of maternally treated fetus with 1/20 LD_{50} of CFP. F. showing decrease in the crown rump length in a dose dependent manner (a) normal maternally controlled fetus (b) maternally treated fetus with 1/20 LD_{50} of CFP (c) maternally treated fetus with 1/10 LD_{50} of CFP.

feti, respectively. The results observed in Table (3) and Figure 1 (d,e,f).

Skeletal Malformations: Feti of treated dams with (1/10 LD50 and 1/20 LD50 of CFP equivalent to 108 mg/kg BW. and 54 mg/kg BW. respectively) showed numerous skeletal malformations in a dose dependent manner in all treated groups (1/10 LD50 and 1/20 LD50 CFP) equivalent to 108 mg/kg BW. and 54 mg/kg BW, respectively) when compared to the control group.

Craniofacial Malformations: The rate of delayed ossification of the skull bone plates of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 72.7% and 26.9% of the total number of examined feti, respectively.

The rate of misshaped skull of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 13.6% and 0% of the total number of examined feti, respectively.

Rib Cage and Vertebral Column Malformations: The rate of delayed ossification of sternbrae of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 63.6% and 19.2% of the total number of examined feti, respectively.

The rate of absence of sternbrae of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 22.7% and 7.7% of the total number of examined feti, respectively.

The rate of delayed ossification of vertebrae of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP

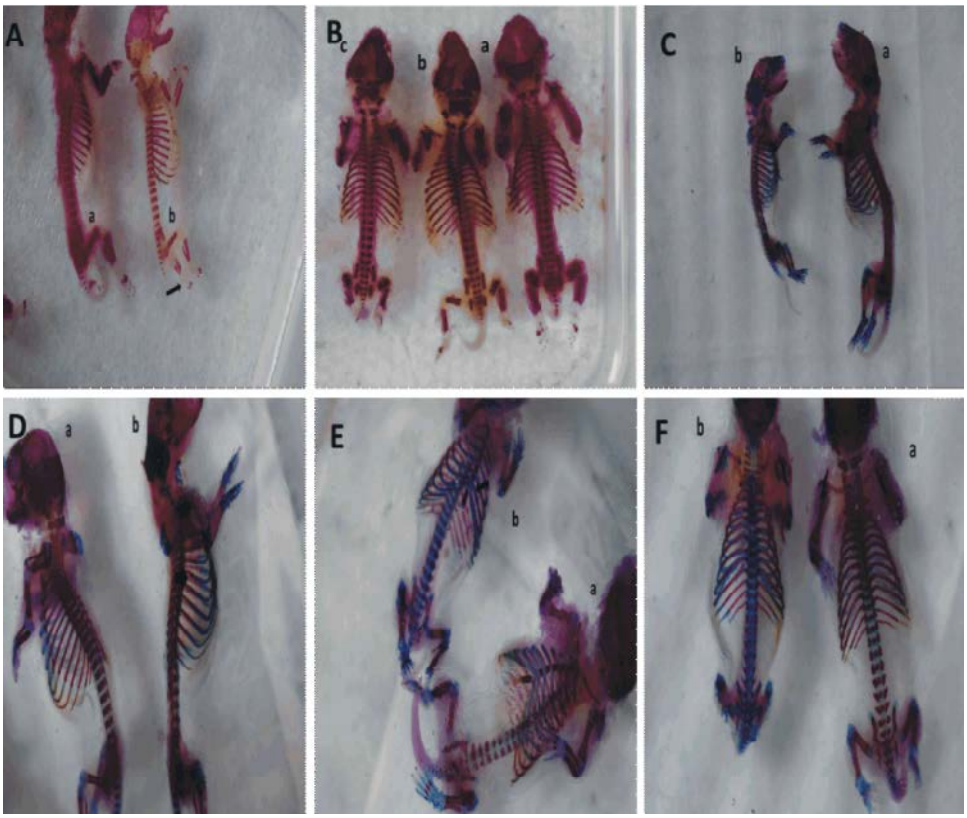


Fig. 2: A. (a) control fetus and (b) maternally treated fetus with 1/10 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed delayed ossification of the phalanges of the hind limb B. (a) control fetus and (b) maternally treated fetus with 1/20 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed delayed ossification of the phalanges of the hind limbs, supraoccipital and interparital bone plates and (c) maternally treated fetus with 1/10 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed sever delayed ossification of the of the phalanges of the hind limbs, supraoccipital and interparital bone plates and caudal vertebrae. C. a) control fetus and (b) maternally treated fetus with 1/10 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed misalignment and failure of fusion of the VII rib to the sternum. D. (a) control fetus and (b) maternally treated fetus with 1/10 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed delayed ossification of centrum and vertebral arch of the thoracic and lumber vertebrae also pelvic bones showed also incomplete ossification. E. showing (a) control fetus and (b) maternally treated fetus with 1/20 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed delayed ossification of the squamosal and frontal bones of the skull. F. showing (a) control fetus and (b) maternally treated fetus with 1/20 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displayed incomplete ossification of the middle portion of the ribs and replaced with cartilagenous plates

rate was about 54.5% and 19.2% of the total number of examined feti, respectively.

The rate of defect in the rib cage of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 36.4 % and 11.5% of the total number of examined feti, respectively.

Appendicular Skeleton Malformations: The rate of delayed ossification of fore limb bones of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 54.5 % and 19.2% of the total number of examined feti, respectively.

The rate of delayed ossification of hind limb bones of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 63.6 % and 23.1% of the total number of examined feti, respectively.

The rate of delayed ossification and absence of the phalanges of feti of treated dams with 108 mg/kg BW. and 54 mg/kg BW. CFP rate was about 77.3 % and 23.1% of the total number of examined feti, respectively. The results observed in Table (4) and Figure 2 (a, b, c, d, e, f).

Table 4: showed rate of skeletal malformations of feti of treated dams with different doses of CFP

Groups	Total number of pregnant rats	Total number of fetuses	Total number of examined fetuses	Skeletal malformations								
				Axial skeleton malformations						Appendicular skeleton malformations		
				Craniofacial malformations		Rib cage and vertebral column malformations						
				delayed ossification of the skull bone plates	misshaped skull	delayed ossification of sternbrae	absence of sternbrae	delayed ossification of vertebrae	defect in the rib cage	delayed ossification of fore limb bones	delayed ossification of hind limb bones	delayed ossification and absence of the phalanges
Control	8	62	31	1 (3.2%)	0	0	0	0	0	0	0	1 (3.2%)
1\10 LD_{50} of CFP	8	43	22	16 (72.7%)	3 (13.6%)	14 (63.6%)	5 (22.7%)	12 (54.5%)	8 (36.4%)	12 (54.5%)	14 (63.6%)	17 (77.3%)
1\20 LD_{50} of CFP	8	52	26	7 (26.9%)	0	5 (19.2%)	2 (7.7%)	5 (19.2%)	3 (11.5%)	5 (19.2%)	6 (23.1%)	6 (23.1%)

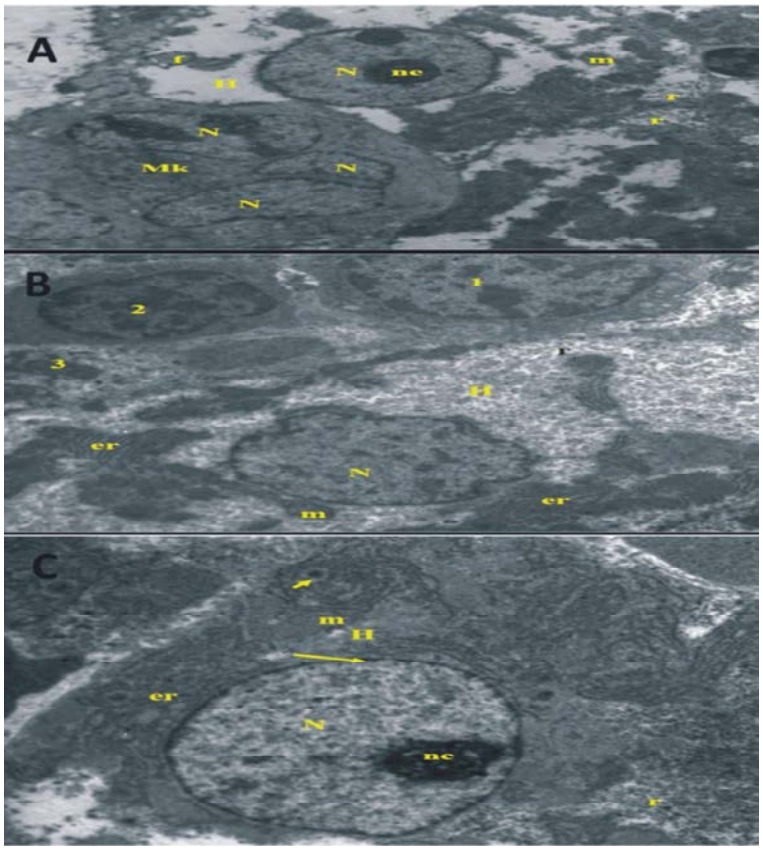


Fig. 3: A. T.E. micrograph of hepatic tissue of maternally controlled fetus displaying presence of lymphoblast and megakaryocyte (Mk) containing abundant cytoplasm and multiple nucleus (N), the hepatic cells (H) having large vesicular nucleus (N) contain two nucleolus (ne) and their cytoplasm contain cell organelles such as free ribosomes (r) mitochondria (m) and fat globules (f). B. T.E. micrograph of hepatic tissue of maternally treated fetus with 1/10 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displaying the hepatic cell (H) having large nucleus (N) contain abundant amount of electron lucent nuclear sap, prominent electron dens nucleolus (ne) and the nuclear chromatin found very thin at the periphery (arrow). The hepatic cytoplasm contains swollen cell organelles such as RER (er) and mitochondria (m) with large number of microbodies (arrow) and free ribosomes (r). D. T.E. micrograph of liver tissue of maternally treated fetus with 1/20 of LD_{50} of CFP from 6th - 15th days of pregnancy daily displaying the hepatic cells (H) contain large vesicular indented nucleus (N) with irregular nuclear membrane and the cytoplasm contain mild swollen RER (er) and mitochondria (m) with large amount of free ribosomes (r), monoblasts (1) or lymphoblsts (2) and blood platelets (3) in the sinusoids are also present in the sinusoids.

Transmission Electron Microscope: The results showed that there was a clear morphological changes in the cellular structure and function of the fetal liver that considered as a hematopoietic organ in the fetal life in a dose dependent manner in the feti of treated dams with different doses of CFP (108 mg/kg BW. and 54 mg/kg BW.) orally from 6th - 15th days of pregnancy daily and the changes were obvious in the higher doses groups in comparison to the control group.

Feti of treated dams with different doses of CFP showed a significant increase in a dose dependent manner in the microbodies and peroxisomes in the hepatocytes besides swelling in the cytoplasmic organelles as mitochondria and RER and abnormal nuclear membrane. The results observed in figure (Fig 3 a, b, c).

DISCUSSION

The results showed that there was a significant increase in fetal death (resorption and still birth), external malformations (subcutaneous hemorrhage or hematoma, hydrocephalus, internal hemorrhage and anomalies of the limbs), visceral malformations (cerebral hemorrhage, dilatation of the ventricles, cleft palate, thickening in the myocardium, abnormal shaped and positioned kidneys besides defect in shape and lobulation of livers and lungs respectively) and skeletal malformations (axial and peripheral skeletal defects) in the treated groups with different doses of CFP (108 and 54 mg/kg BW.) in a dose dependent manner in respect to the control group. In contrast to our finding, Schneider [16] found that different concentration of chlorfenapyr showed no visceral or skeletal malformation during fetal observation, However moderate vaculation in the brain white matter was observed microscopically with a slight movement depression.

Up withstanding to our results, firstly, placental transfer of pesticides is affected by different factors related to pesticide as degree of ionization, lipid solubility and molecular weights or to placenta itself as placental age, maternal blood flow and placental metabolism, chemicals have a molecular weight below 600 can transmigrate from the placenta so chlorfenapyr capable of transporting through the placenta [17]. Secondly, the lipid peroxidation tends to increase in the fetal than maternal tissues in spite of the presence of the placenta that naturally protect the fetus against free radicals as a selective transporter but the defense system may be overwhelmed by high level of ROS [18]. Thirdly, Chlorfenapyr act through interfering with the oxidative phosphorylation process in the mitochondria and deprivation of cellular energy and production of free radicals causing oxidative stress and significant increase in ROS production [19]. Fourthly, the decrease fetal numbers and embryonic death in treated groups may be because of the decrease of oval production and incomplete placental formation besides DNA damage either partial or complete and transcription inhibition in the highly active fetal cells respectively due to maternal toxicity, partial damage in the DNA resulted in malformations while complete damage resulted in embryonic death [20]. Fifthly, visceral abnormalities as ventricular dilatation and abnormal shaped liver and kidney may be attributed to lack of the placental transfusion nutrients as amino acid, arginine and defective fetal metabolism [21]. Sixthly, delayed ossification considered also as an indicator of growth retardation and the uncouplers of the oxidative phosphorylation process as chlorfenapyr depress the ATP synthesis with subsequent deprivation of the embryo from the energy source that is vital to the fetal growth and development [22].

Several hemorrhagic spots or subcutaneous hemorrhage had been observed and cause may be due to the platelet dysfunction that may occurred due to the interference of cellular ADP release that initiate the platelet aggregation cascade necessary in the coagulation process furthermore the defect in the coagulation system may also be due to the hepatoxicity and liver injuries induced by pesticides with subsequent disturbances in the circulating coagulation factors and defect in the coagulation cascade [23,24].

The ultrastructural microscopical examination of embryonic liver that considered as hematopoietic organ in the fetal life revealed that was a clear morphological changes in the cellular structure and function of feti of treated dams with different doses of CFP (108 and 54 mg/kg BW. CFP) in a dose dependent manner in respect to the control group.

For feti of treated dams with different doses of CFP there was a significant increase in a dose dependent manner in the microbodies and peroxisomes in the hepatocytes besides swelling in the cytoplasmic organelles as mitochondria and RER and abnormal nuclear membrane.

Peroxisomes appear in the liver if rats on the day 14^{th} or 15^{th} of gestation and contain many enzymes that mainly responsible for lipid metabolism and â oxidation of the fatty acids and proliferation of peroxisomes occurred under certain stimuli as exposure to pesticides with

excessive ROS production and lipid peroxidation so increase in the peroxisomes usually considered as a biomarker of oxidative stress besides mitochondrial and RER swelling reflecting cellular degeneration with excessive highly reactive metabolites production [25]. Feti were often susceptible to toxicant due immature developmental state, lack of adequate defense mechanisms, transplacental passage of pesticides and its effects of DNA synthesis [26] could enhanced teratogenicity of that chemicals [27]. Finally, there is scientific correlation between geno-toxicity of Chlorenapyr [28] and its teratogenicity. The current finding support the evidence of link between maternal work in greenhouses and spontaneous abortion [29].

CONCLUSIONS

Chlorenapyr an insecticide used in greenhouses in Egypt but different doses of Chlorenapyr could increase the external, visceral, skeletal malformations and alteration tissue ultrastructure.

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