Short communication

Hepatorenal effects of chlorfenapyr as pro-insecticide in female Sprague Dawleyrats in postnatal model of toxicity and in HepG2 cell line.

Mahmoud M. Elalfy[1], Mohamed S. Abomosallam[1], Fathy R. Sleem[1], Emad A. Abassand Mona G. Elhadidy[2]

ABSTRACT: Chlorfenapyr is good candidate insecticide for control of vectors blood borne diseases like malaria. As little information about Chlorfenapyr toxicity and possibility of its residue's presence in food stuffs, milk and environment, we explore the postnatal toxic effects of Chlorfenapyr in female Sprague dawley rats and its pups. Chlorfenapyr was given orally at doses of 0,54 and 108 mg/kg to female albino rats immediately at first day after delivery till 21 days of lactation. All dams and its pups were weighted, euthanized and blood was separated for serum separation and tissues were preserved either at 4c as tissue homogenate for measurements of oxidant/ antioxidants levels or in buffered formalin for histopathological examination. The highest dose of Chlorfenapyr induced hepatorenal toxicity in dams and its pups with evidence of increase liver enzymes and creatinine level when compared to control groups. Also,Chlorfenapyr displayed histopathological changes in liver, kidney, brain and spleen tissues of dams as well as rats' pups after 21 days of treatment. The toxic effects resulted from secretion of Chlorfenapyr in milk and increased the free radicals' production and oxidants like MDA in tissues of rats pups. Also, chlorfenapyr had a cytotoxic effects on HepG2 cells indicated by induction of oxidative stress and lethality to cell line.. Taken collectively, chlorfeapyr is a good candidate insecticide in vector control but had a cytotoxic effects in female albino rats, its pups, and in HepG2 cells

Key words: chlorfenapyr, pro-insecticide, cytotoxic effects, oxidative stress, albino rats, HepG2 cells

INTRODUCTION

SChlorfenapyr(CFP) is a pro-insecticide used since 1995for control of agriculture pest. Human toxicity from CFP was little as only few cases were reported withnausea, vomiting, fever, rhabdomyolysis (1-4) and nervous system toxic manifestation (5) after ingestion of current insecticide.

Chlorfenapyr is a member of a new class of insecticide, of pyrroles group (chemical name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile; trade name: Pylon miticide-insecticide)(6). The uses of the CFPwere removal of mites, caterpillar pests, thrips, and fungus gnats by foliar spray on ornamental crops in greenhouses and its mode of action was throughinhibition of oxidative phosphorylation in the mitochondria, resulting in reduction of ATP production, cellular death, and ultimately, death of the organism(7).

The Chlorfenapyr is a light tan or light-yellow solid powder. While CFPtoxicity has not yet been studied in humans and animals resulted that classification of CFPtoxicity as category III chemical. Recently, In few studies recorded that CFP induced developmental and maternal toxicity in female albino rats (7, 8).

Chlorfenapyr sources or residues were recorded in environment (9, 10), in food products(11-13) , water(14), animal derived foods(15) and in tissue of treated rats(8)analyzed by different methods of chromatography like GLC and HPLC. The rationale of this study to explore the postnatal toxic effects in female albino rats and its pups.

Materials and methods

Animals

Sprague Dawley rats obtained from 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 then adapted for about 2 weeks and maintained on a balanced ration before the experiment.

Tested chemicals

Chlorfenapyr is a light green wettable powder (WP) with slight chlorine odor and kindly obtained from Central Agricultural Pesticide Laboratory, Ad Doki, Giza, Giza Governorate after HPLC analysis to confirm the percentage. HepG2 (85011430, sigma, USA) was gifted to us was provided to us from faculty of medicine, Mansoura university, Egypt

Experimental design

Eighteen (18) pregnant female Sprague Dawley rats were separated into three groups with six females for each.Chlorfenapyr was given orally and daily at doses of 0,54 and 108 mg/kg (equivalent to 1/20 and 1/10 of LD50) to female albino rats immediately at first day after delivery till 21 days of lactation. the neonates litter size recorded and both dams and neonates weighed daily and kept under observation until weaning (21 day postpartum) the day of sacrifice

Clinical signs

The treated dams and neonates observed daily throughout the experimental period for any abnormal behavior, findings or alteration.

Maternal and neonatal body weight gain

The initial body weight determined from the day of parturition for both dams and neonates and then throughout the experiment the body weight calculated before each administration. The body weight gain % determined according to the following formula (16).

Body weight gain %=(Final body wt – initial body weight)/(initial body weight) ×100

1.Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Mansoura University, Egypt.
2. Medical physiology, Faculty of Medicine, Mansoura University, Egypt.
3.Pharmacology department, Faculty of Medicine, Mansoura University, Egypt. Postgraduate researcher form Iraq

Correspondence: Mahmoud Elalfy Forensic medicine and toxicology department, faculty of veterinary medicine, Mansoura University, 35516, Mansoura, Egypt, E-mail: : mahmoudelalfy@mans.edu.eg

Sample collection

At day 21 postpartum, dams and weaned rats 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 contain K3EDTA as anticoagulant.

The fresh blood collected in gel tube (not contain anticoagulant) for serum separation in centrifuge at 3000 rpm for 15 minutes then stored at-20°C. Also, the liver tissue sample removed from dams and weaned rats and washed with saline solution then one gram of tissues 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°C , the supernatant separated, collected and stored at-20°C in Eppendorf tubes (17).

Theliver, kidney, spleen and brain specimen from both dams and weaned rats collected and kept in 10% neutral buffered formalin for histopathlogical processing and analysis,.

Hematological examination

Blood sample analysis 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) evaluated besides total and differential white blood cells were also measured (18).

Biochemical analysis

Gamma glutamyl transferase activity, Alanine aminotransferase activity, Glucose, serum total protein, albumin, creatinine, urea and cholesterol level were measured in serum of treated and control group.

Antioxidant and oxidative stress biochemical analysis

Liver homogenate of all treated groups and pups were analyzed for GSH, GST, SOD, CAT and MDA levels.

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

Cytotoxicity of chlorfenapyr on HepG2 cells

The stock solution of chlorfenapyr (100 mmol/L) was prepared in ethanol and stored at 4°C. Working solutions were prepared by dissolving the stock solution in the culture medium. We exposed HepG2 cells to different concentrations (0, 10,20, 40 ng/mL) of chlorfenapyr for 24 hours to determine its toxic effects. The HepG2 cells were cultured according methods described earlier(19)

cell viability and oxidative stress tests

The viability of cells were detected by quantification of formed formazan salt. In this regard, HepG2 cells (2 × 105 cells) were seeded in 96-well plates. Later 24 h, the medium solution was changed with other medium containing different concentrations (0, 10, 20,40 ng/mL) of chlrfenapyr and solvent (ethanol) were added for 24. MTT (50 µg/mL) was added to each well. After 4 h incubation at 37 °C, the later solution was discarded and formed formazan crystals was dissolved in DMSO (100 µL). The color developed was measured at 570 nm using a multiplate reader (Synergy HT, Bio-Tek, Winooski, Vermont).

Additionally, the cell extract was centrifuged (10000 g, 10 min, 4°C) and supernatant was used for oxidative stress assays such as glutathione (GSH), superoxide dismutase (SOD), and MDA

Statistical analysis

Statistical analyses were carried out using SPSS software program (13, USA). Homogeneity of the groups were tested by Kruskal Walis test. one-way ANOVA was used to define significance between groups at $p< 0.05$ (20).

Results

Postnatal maternal and pups' body weight gain% upon exposure to CFP.

A relative significant decrease in the body weight gain in all treated group, lactating dams throughout the study in respect to the control group especially groups of 1/10 LD50 ofCFP results illustrated in table 1. Additionally, there was a significant decrease in the body weight gain of pups in all treated groups in respect to the control group and results illustrated in table 1.

TABLE 1

The initial and final body weight mean and body weight gain % in lactating female rats administered orally different doses of chlorfenapyr postnatally from 0th to 21th days postpartum daily in comparison to the control group (mean ± SE).

Group	Mean Initial body weight	Mean Final body weight	Body weight gain %
Group 1 (Control)	158.67±2.33	206.67±0.88a	30.25
Group 2	156.67±2.03	180.23±1.53e	15.04
Group 3	158.21±0.58	189.67±1.76c	19.88

TABLE 2

The initial and final body weight mean and body weight gain % in pups of maternally treated dams orally with different doses of chlorfenapyr postnatally from 0th to 21th days postpartum daily in comparison to the control group (mean ± SE).

Group	Mean Initial body weight	Mean Final body weight	Body weight gain %
Group 1 (Control)	5.47±0.12	34.83±0.73a	536.75
Group 2	5.37±0.29	24.13±0.58c	349.35
Group 3	5.87±0.12	30.67±0.88b	422.49

Postnatal maternal and pups' biochemical analysis

Metabolic, liver and kidney functions' biomarkers in lactating dams and pups

a) Metabolic, liver and kidney functions' biomarkers in lactating dams

In lactating dams, the results showed that a significant decrease in glucose, cholesterol and total protein in all treated groups in respect to the control group especially group of 1/10 LD50 of CFP equivalent to 108 mg/kg Bw. Also, the group of 1/10 LD50, 1/20 LD50 of CFP equivalent to 108 mg/kg Bw. and 54 mg/kg Bw respectively showed a significant decrease of albumin and globulin levels in comparison to control group (table 3).

TABLE 3

The postnatal maternal biochemical metabolic, liver and kidney biomarkers changes after administration of different doses of CFP orally from 0th - 21th days postpartum daily in comparison to the control group

	ALT (U/l)	AST (U/l)	GGT (U/l)	Glucose (mg/dl)	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin	Group	Group
Control	24.06±1.97c	34.67±1.7d	16.63±2.49c	152.47±2.66a	93.9±1.84a	8.53±0.52a	5.77±0.45a	2.77±0.28a	39.33±0.85c	0.56±0.02b
Group 1/10 of CFP LD50	44.14±2.04a	64.4±2.46b	37.01±2.86a	92.77±2.83c	72.8±3.59c	4.93±0.09c	3.32±0.63cd	1.61±0.31b	67.97±4.36a	1.33±0.23a
Group 1/20 CFP of LD50	34.5±2.47b	40.1±1.14cd	25.23±3.19b	143.03±0.9b	74.13±2.58c	7.07±0.21b	4.32±0.78bc	2.73±0.23a	54.29±3.32b	0.72±0.04b

In table three, there was a significant increase of all biomarkers (ALT, AST and GGT) in most of treated groups in comparison to the control group especially higher doses groups (1/10 LD50 of CFP equivalent to 108 mg/kg Bw.). In addition, there was a significant increase in blood urea nitrogen in most of treated groups in comparison to the control group especially groups 1/10 LD50 of CFP equivalent to 108 mg/kg Bw. On the other hand,only in groups 1/10 LD50 of CFP equivalent to 108 mg/kg Bw. showed a significant increase creatinine level.

b) Liver and kidney functions' biomarkers in pups:

Liver and kidney functions estimated in pups of maternally treated dams for the same groups as illustrated in table (4).

In table four, there was a significant increase in ALT and GGT in all maternally treated groups in comparison to the control group especially maternally treated groups of 1/10 LD50 of CFP equivalent to 108 mg/kg Bw. Also, there was a significant increase in blood urea nitrogen and creatinine in all treated groups in comparison to the control group especially group of 1/10 LD50 of CFP equivalent to 108 mg/kg Bw

TABLE 4

The biochemical liver and kidney biomarkers changes in maternally treated pups with different doses of CFPorally from 0th - 21th days postpartum daily in comparison to the control group.

	ALT (U/l)	GGT (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control	35.27±1.22^{c}	24.30±0.96^{c}	47.33±1.65^{c}	0.69±0.02^{c}
Group 1/10 of CFP LD50	60.74±3.73^{a}	53.97±2.92^{a}	77.62±2.12^{a}	1.82±0.08^{a}
Group 1/20 CFP of LD50	44.43±2.47^{b}	33.34±1.44^{b}	59.02±1.56^{b}	0.86±0.04^{b}

Oxidative stress biomarkers in lactating dams and pups

a) Oxidative stress biomarkersin lactating dams

The lactating dams shown a significant decrease in GSH, GST and CAT in most of treated groups when compared with the control group especially higher doses groups (1/10 LD50 of CFP equivalent to 108 mg/kg Bw. The treated groups (1/10 LD50 CFP equivalent to 108 mg/kg Bw. showed significant decrease in level of SOD. All treated groups showed a significant increase MDA oxidant in respect to the control group especially groups of 1/10 LD50 of CFP equivalent to 108 mg/kg Bw.

TABLE 5

The biochemical oxidative stress biomarkers changes in lactating dams after administration of different doses of CFP group orally from 0th - 21th days postpartum 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
Group 1 (Control)	28.69±0.95^{a}	10.78±0.81^{a}	27.42±1.04^{a}	17.96±0.94^{a}	30.97±0.93^{c}
Group 2	15.91±1.42^{b}	6.05±0.47^{c}	16.08±1.53^{b}	10.94±0.71^{c}	73.96±1.90^{a}
Group 3	19.67±1.29^{b}	7.69±0.44^{b}	24.82±1.39^{a}	13.54±0.76^{b}	61.14±2.72^{b}

TABLE 6

the biochemical oxidative stress biomarkers changes in pups of maternally treated dams with different doses of CFP orally from 0th - 21th days postpartum 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
Group 1 (Control)	18.94±1.10^{a}	8.04±0.44^{a}	14.63±1.17^{a}	9.37±0.51^{a}	49.45±0.96^{c}
Group 2	10.11±0.39^{c}	4.37±0.45^{b}	8.74±1.13^{b}	5.49±0.29^{b}	87.38±1.03^{a}
Group 3	14.33±1.06^{b}	5.53±0.29^{b}	13.73±0.64^{a}	8.84±0.71^{a}	69.29±0.65^{b}

b)Oxidative stress biomarkersin pups

Thepups of maternally treated dams displayed a significant decrease in GST, GSH, SOD and CAT in most of treated groups when compared with the control group especially higher doses groups (1/10 LD50 of CFP equivalent to 108 mg/kg Bw. On the other hand, all maternally treated groups in respect to the control group especially groups of 1/10 LD50 of CFP equivalent to 108 mg/kg Bw. showed a significant increase in MDA level when compared to control group.

Hematological Finding in lactating dams

There was a significant decrease in the Hb content in the groups received 1/10 LD50 of CFP equivalent to 108 mg/kg Bw in comparison to the control group. Also, total leukocytic count showed a significant increase in the groups treated with 1/10 LD50 of CFP equivalent to 108 mg/kg

TABLE 7

The hematological finding in lactating dams after administration of different doses of CFP orally from 0th - 21th days postpartum 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)
Group 1 (Control)	8.42±0.02^{a}	14.76±0.05^{a}	45.75±0.05^{a}	54.34±0.20	17.53±0.10	32.26±0.10	7.67±0.10^{b}
Group 2	8.22±0.15^{a}	14.22±0.12^{b}	44.94±0.03^{a}	54.68±0.97	17.32±0.22	31.65±0.25	8.82±0.07^{a}
Group 3	8.42±0.14^{a}	14.56±0.10 ^{ab}	45.76±0.10^{a}	54.36±0.98	17.30±0.16	31.83±0.28	7.63±0.17^{b}

Bw. in comparison to the control group. On the other hand, there was no significance change in all treated groups in PCV, MCV, MCH and MCHC levels in respect to the control values.

Histopathological findings

The histopathological changes were observed in lactating dams and pups of maternally treated dams with different doses of CFP (108 mg/kg Bw. and 54 mg/kg Bw.) in comparison to the control group and the results showed that there was severe pathological changes especially at the higher doses groups

Liver

The lactating dams treated with different doses of CFP displayed degenerative changes and intralobular histiocytic infiltration with intralobular fibroblastic proliferation in the hepatic tissue. While the pups of treated dams with different doses of CFP shown a focal histiocytic and lymphocytic infiltration besides congestion of portal vein and margination of leukocytes in a dose dependent manner in respect to the control group, results illustrated fig 1

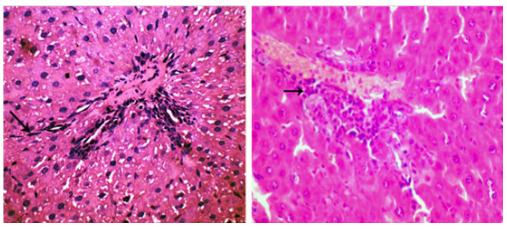

Figure 1) *The depicted figure shown (a) Liver of lactating dams treated with 1/10 LD50 of CFP orally from 0th - 21th days postpartum showing intralobular fibroblastic and histiocytic infiltration in the hepatic tissue (arrow) in (HE, 400x). (HE, 400x) (b) Liver of suckling pups of treated dams with 1/10 LD50 of CFP orally from 0th - 21th days postpartum showing mild lymphocytic infiltration in hepatic tissue (arrow) (HE, 400x).*

Brain

The lactating dams treated with different doses of CFP there was neuronal necrosis, neuronophagia and astrocytosis in the brain tissue with degenerative changes of purkinje cells in cerebellum and the pathological changes were more sever in respect to the control group, results illustrated in fig2 (a,b)

Thepups of treated dams with different doses of CFP there was focal edema in the neutrophils with focal hemorrhagic areas in the brain tissue in a dose dependent manner in respect to the control group, results illustrated in fig2 (c).

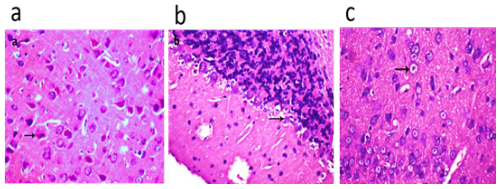

Figure 2) *The depicted figure shown (a) Brain of lactating dams treated with 1/10 LD50 of CFP orally from 0th - 21th days postpartum showing central chromatolysis and neuronal necrosis in the brain tissue (arrow) (HE, 400x) (b) Brain of lactating dams treated with 1/20 LD50 of CFP orally from 0th - 21th days postpartum showing degenerative changes of purkinje in cerebellum (arrow) (HE, 400x) (c) Brain of suckling pups of treated dams with 1/10 LD50 of CFP orally from 0th - 21th days postpartum showing cytotoxic edema of neurons (arrow) in (HE, 400x).*

Kidney

The lactating dams treated with different doses of CFP displayed fibrous tissue proliferation of renal glomeruli with degenerative changes in renal tubular epithelium results illustrated in fig 3 (a). while the pups of treated dams with different doses of CFP showna degeneration in the renal tubular epithelium and interstitial lymphocytic infiltration with congestion of the renal glomeruli in a dose dependent manner in respect to the control group, results illustrated in fig 3 (b).

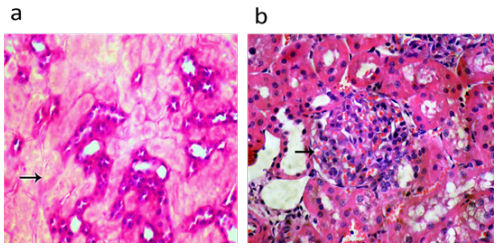

Figure 3) *The depicted figure shown a) kidney of lactating dams treated with 1/10 LD50 of CFP orally from 0th - 21th days postpartumshowingdegenerative changes and necrosis of renal tubular epithelium (HE, 400x). (b) kidney of suckling pups of treated dams with 1/10 LD50 of CFP orally from 0th - 21st days postpartum showing congestion of renal glomeruli (arrow) (HE, 400x).*

Spleen

For lactating dams treated with different doses of CFP there was marked lymphoid depletion in the splenic tissue and the pathological changes were more sever in the dose of highest dose of CFP in respect to the control group, results illustrated in fig 4 (a).

The pups of treated dams with different doses CFP displayeda sever lymphoid depletion with congestion of the splenic sinusoids in a dose dependent manner in respect to the control group, results illustrated in fig 4(b).

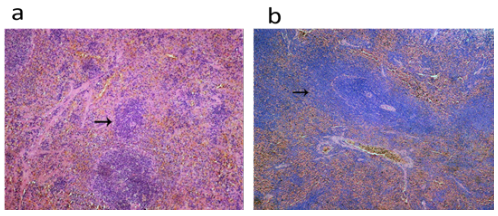

Figure 4) *The depicted figure shown (a) Spleen oflactating dams treated with days postpartumshowing severe depletion of the splenic lymphoid tissue (arrow) (HE, 100x). (b) Spleen of suckling pups of treated dams 1/10 LD50 of CFP orally from 0th - 21th days postpartum showing lymphoid depletion (arrow) (HE, 400x).*

Notably, the chlorfeapyr treatment to hepG2 cells at highest concentration of clorfenapyr at 20 and 40 is cytotoxic as increased numbers of dead cells versus viable hepG2 cells figure 6. Moreover, the cell lysis shown a significant reduction of glutathione superoxide dismutase, and increased MDA level especially at high concentration of chlorfenpayr at doses of 20 and 40 ng/ml (table 8).

TABLE 8

effect of chlorfenapyr on oxidative stress in (HepG2) cells

Lo0	GSH mg/g. tissue	SOD U/g. tissue	MDA nmol/g. tissue
0	20.69±1.095[a]	8.42±0.054[a]	14.97±0.93[c]
10 ng/ml	18.69±1.09[a]	7.42±0.034[a]	13.97±0.93[c]
20	10.69±1.03[b]	3.42±0.054[b]	33.97±0.93[b]
40	8.91±1.042[b]	2.08±0.53[b]	75.96±1.90[a]

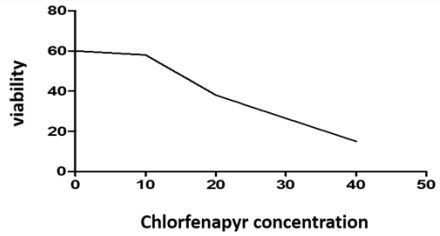

Figure 6) *showed that chlorfenpayr at high concentration induce lethal action to hepG2 cells*

Discussion

Chlorfenapyrhasa household and agriculture uses but also have a hazardouseffects on animals and human health (1-4) especially when contaminated the environment (9, 10), or remained as residues in food products (11-13) , water (14), animal derived foods (15).

Chlorfenapyr, a pyrrole insecticide has displayed a potential role to control of parathyroid resistant insects (21) and it was consider as a candidate insecticide for targeting malaria vectors that were resistant to pyrethroids (22). Even chlorfenaapyr considered a good candidates insecticides, it had developmental and maternal toxicity reported earlier (7, 8).

Here, we are the first report of postnatal effects of chlofenapyr in female albino rats and its pups through hepatorenal toxicity, increased the oxidant, reduction of antioxidant levels in tissue of dams and pups and pathological changes in both tissue of dams and its pups. In this regard, chlorfenapyr could pass through placental barrier as it evidence its presence in milk as residues (15). In consistent to current study, maternal toxicity of oral exposure chlorfenapyr at doses of 1/10 , 1/20 of LD50 in female albino rats recorded earlier with evidence of induction of pathological features in liver, kidney, placenta, increased activity of liver enzymes, urea and creatinine levels, MDA as oxidant and reduced the levels of antioxidants (8). Notably, chlorfenapyr

induced a significant inhibition in the activity of GST in the antioxidant in CHOK1 cells which retain GST levels after treatment with vitamin C or vitamin E(23). Additionally, Chlorfenapyr had a cytotoxic effect in the different antioxidant assays. Chlorfenapyr is a pyrrole pro-insecticide; it is bio transformed by phase 2 oxidative elimination of N-ethoxymethyl group, which induced ablation of themitochondrial ATP production through uncoupling of the mitochondrial oxidative phosphorylation that might enhanced the reactive oxygen radicals production (23, 24).Notably, the cytotoxic effect of chlorfenapyr recorded on CHO-K1 cell line (23)as well as indicated cases of human intoxicated with chlorfenapyr (25).

In conclusions, chlorfeapyr is a good candidate insecticide in vector control but had a cytotoxic effects in female albino rats, its pups, and in HepG2 cells

All authors have no conflict of interest

Acknowledgments

All authors thank dr Mohamed Fawzy for help us in pathological examination and comments of all lesion in this research work. In addition, we thank for all staff member of our department for support us during experimental research. Medicine.

REFERENCES

1. Kang C, Kim DH, Kim SC, Kim DS. A patient fatality following the ingestion of a small amount of chlorfenapyr. Journal of emergencies, trauma, and shock. 2014;7(3):239.

2. Endo Y, Tachibana S, Hirano J, Kuroki Y, Ohashi N, Yoshioka T, et al. Acute chlorfenapyr poisoning. 2004.

3. Hoshiko M, Naito S, Koga M, Mori M, Hara K, Ishitake T. Case report of acute death on the 7th day due to exposure to the vapor of the insecticide chlorfenapyr. Chudoku kenkyu: Chudoku Kenkyukai jun kikanshi= The Japanese journal of toxicology. 2007;20(2):131-6.

4. Choi JT, Kang GH, Jang YS, Ahn HC, Seo JY, Sohn YD. Fatality from acute chlorfenapyr poisoning. Clinical Toxicology. 2010;48(5):458-9.

5. Kwon J, Kim H, Han H, Kim J, Park J. A case of chlorfenapyr intoxication with central nervous system involvement. J Clin Toxicol. 2012;2(8):147.

6. Lee J, Lee JH, Baek JM, Lee DS, Park IY, Won JM, et al. Toxicity from intra-abdominal injection of chlorfenapyr. Case reports in emergency medicine. 2013;2013.

7. Sleem F, Elalfy MM, Allah AAA, Hamed MF, Abomosallam M. Developmental and Ultrastructure Toxicity of Greenhouse Insecticide Chlorfenapyr in Rat Fetuses. 2019.

8. Elalfy M, Abomosallam M, Allah A, Mahmoud H, Eleuony O. Maternal and Cytotoxic Effects of Chlorfenapyr as a Pro-Insecticide in Pregnant Female Albino Rats. 2019:29-38.

9. Cao Y, Chen J, Wang Y, Liang J, Chen L, Lu Y. HPLC/UV analysis of chlorfenapyr residues in cabbage and soil to study the dynamics of different formulations. Science of the Total Environment. 2005;350(1-3):38-46.

10. Mai Y-L, Zhong G-H, Hu M-Y, Liu X-Q. Determination of chlorfenapyr residues in cabbage and soil by gas chromatography. PESTICIDES-SHENYANG-. 2004;43(5):233-5.

11. MAI Y-l, LIU H-m, LIU C-l, CHEN W-t, HU M-y. Study on Residue Dynamics of Chlorfenapyr in Cucumber and Amaranth [J]. Journal of South China Agricultural University. 2007;1.

12. WU D, LUO X-t, PAN H-j, NIE X-y, FAN L-j. GC determination of chlorfenapyr residue in cucumber s and apples. Food Science. 2010;31(10):268-71.

13. Huang-Fu W-g, Chen G, Yang T. Residue detection of chlorfenapyr in vegetables. Acta Agriculturae Zhejiangensis. 2007;19(2):119.

14. Wu Y, Zhou Z. Application of liquid-phase microextraction and gas chromatography to the determination of chlorfenapyr in water samples. Microchimica Acta. 2008;162(1-2):161-5.

15. Hamilton DJ. A data-fitted mathematical sequence to calculate depuration rates for pesticide residues in milk from dairy cow feeding studies. Computational Toxicology. 2018;6:1-8.

16. Bhardwaj S, Srivastava M, Kapoor U, Srivastava L. A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. Food and chemical toxicology. 2010;48(5):1185-90.

17. Fernandez-Botran R, Gorantla V, Sun X, Ren X, Perez-Abadia G, Crespo FA, et al. Targeting of glycosaminoglycan-cytokine interactions as a novel therapeutic approach in allotransplantation1. Transplantation. 2002;74(5):623-9.

18. Agbasi P. U. AN, Onye J. J., Ibeawuchi C., Uzoechi S. C., Alagwu E. A., Okeke C.U., Uloneme G. C. . The effect of subchronic low dose of DDVP and sodium azid on the hematological indices of albino rats. toxicology physiology and biochemistry. 2015;4:103-10.

19. AlKahtane AA, Alarifi S, Al-Qahtani AA, Ali D, Alomar SY, Aleissia MS, et al. Cytotoxicity and genotoxicity of cypermethrin in hepatocarcinoma cells: a dose-and time-dependent study. Dose-Response. 2018;16(2):1559325818760880.

20. Snedecor GW, Cochran WG. Statistical Methods, eight edition. Iowa state University press, Ames,Iowa. 1989.

21. Ngufor C, N'Guessan R, Boko P, Odjo A, Vigninou E, Asidi A, et al. Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant Anopheles gambiae: an experimentalhut trial in Benin. Malaria journal. 2011;10(1):343.

22. N'guessan R, Boko P, Odjo A, Akogbeto M, Yates A, Rowland M. Chlorfenapyr: a pyrrole insecticide for the control of pyrethroid or DDT resistant Anopheles gambiae (Diptera: Culicidae) mosquitoes. Acta tropica. 2007;102(1):69-78.

23. Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO K1 cells. Environmental Science and Pollution Research. 2015;22(21):17041-52.

24. Black BC, Hollingworth RM, Ahammadsahib KI, Kukel CD, Donovan S. Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles. Pesticide Biochemistry and Physiology. 1994;50(2):115-28.

25. Chomin J, Heuser W, Nogar J,Ramnarine M, Stripp R, Sud P. Delayed hyperthermia from chlorfenapyr overdose. The American journal of emergency medicine. 2018;36(11):2129. e1-. e2.