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STUDY SOME PHYSIOLOGICAL ASPECTS AND HISTOPATHOLOGICAL CHANGES IN MALE RABBITS INTOXICATED BY CHLORPYRIFOS

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Abstract:

The current experience on chlorpyrifos was undertaken to investigate the potential alterations in Physiological Criteria and in liver and kidney tissues subsequent oral exposure of chlorpyrifos in male rabbits . twelve of male rabbits were employed in this experience, distributed into two groups randomly , group one received only corn oil and served as control, whereas, while group two received CPF 33.3 mg/kg dissolved with(1ml) corn oil daily, the exposure continued for 28 consecutive days orally. outcomes of this work revealed that sub-acute oral administration of chlorpyrifos cause to oxidative stress evidenced by pronounced elevation value of oxidative biomarker malondialdehyde (MDA) concomitantly with elevated in values of inflammatory biomarkers (TNF-α and CRP) and significantly reduction in total antioxidant capacity (TAC) value, as well as this findings exhibited that chlorpyrifos administration cause to marked alteration of blood indices values as well as histopathological changes were observed in liver and kidney which as represented degenerative of hepatocyte, dilatation and congestion in central vein, While tubular epithelial degenerations, vacuolations and severe dilation in renal cortex tubules. Conclusion: we concluded from findings in this study the oral exposure to CPF evoked inflammation response and oxidative damage as represented by pronounced elevation in levels of CRP,TNF-alpha and MDA biomarkers as well as pathohistological alterations were observably in liver and kidney that indicating that chlorpyrifos elicits numerous adverse effects noteworthy making it a major public health issue.

Keywords: Chlorpyrifos, , CRP , TNF-A, CBC, TAC And Oxidative Index.

Introduction:

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Organophosphorous (OPS) are pesticides that extensively used in agriculture because its effectiveness in controlled on insects and protect the crops food, animals and humans being a part of ecosystem are constantly exposed to these chemicals through environmental contamination (Burke, 2016and Juntarawijit and Juntarawijit,2018). In today, the Pesticides have greater attention due to contamination emerged as a hazard problem worldwide this due to popularity and prevalence use (Donkor *etal.*,2016). Animals and humans can be exposure to pesticides by either directly through the inhalation and skin contact and by the consumption of contaminated food indirectly (Bon *etal.*,2014 and Kutluyer *etal .,* 2017). In recently, numerous reports revealed deleterious effects of ROS (free radicals) and their a major role in provoked toxicity of pesticides, exposure to pesticides produces oxidative damage in the cells by over- production oxygen free radicals (ROS) that exhaust and consume antioxidant agent within cells(Lukaszewicz-Hussain.2010and Abdel Aziza *etal.*,2021).

Chlorpyrifos (C9H11CI3NO3PS; O,O-diethylO-3,5,6-trichloro-2-pyridyl phosphorothioate; CPF). Chlorpyrifos is CPF) is one most of organophosphorus pesticides which used to control pests in the crops and in other professional settings as veterinary uses, domestic uses, wood treatment, maintenance of green spaces and disinfection of premises, it is broad-spectrum(Sharma and Chadha, 2016). CPF principally acts as an acetylcholinesterase (AChE) inhibitor, the cytochrome P-450 drug-oxidizing system activated it to chlorpyrifos Oxon(CPF-O), its main metabolite, which is the responsible for the inhibitory impact of CPF on acetylcholinesterase resulted in neurological disorders (Saoudi *etal.*,2018 and Mahmoud *etal.*,2019) . Exposure to CPF reportedly elicits toxicity through disturbances of cytokine balance and provoked oxidative stress are which contribute to health hazards (Dominah *etal.*,2017) . Chlorpyrifos cause to disrupting effects as teratogenicity, embryo- and geno- toxicity, weakened immune and imbalance antioxidant defense system, neurobehavioral alterations, endocrine disturbance, reproductive toxicity, and hepato and -nephro toxicity (Albuquerquea *etal.*,2017). This work was aimed to evaluate infammation indices and oxidative index and their correlation with the adverse effects of chlorpyrifos on blood indices, liver and renal histoarchitecture in male rabbits.

Materials and methods

Chemicals

The chlorpyrifos :(CPF; O, O-diethyl-O-3, 5, 6-trichloro-2-pyridyl phosphorothioate; Molecular formula; C9H11Cl3NO3PS, chlorpyrifos 98% purity) white powder, it was obtained from company of chemical, seeds and agricultural products (Sigma R Chemicals Company).

Experimental protocol

Twelve of male domestic rabbits with weighing between 1200-1300g were used in this experiment, these animals were brought from local market of Basra city, four rabbits were housed in each cage in the standard condition (23 ± 2° C, 12h light/ dark) in the animals house of College of Veterinary Medicine, University of Basra, Iraq and acclimatized at 7 days prior study starting , the diet freely also tap water was supplied *ad libitum* throughout the experimental period. At end the duration of adaptation the rabbits were randomly distributed into two groups, each group consist of 6 animals represent an experimental group and control group as follows:. Intact animals served as a control were administered (1 ml) corn oil orally for 28 consecutive days, while Experimental group were exposed to CPF of 33.3 mg/kg dissolved with(1ml) corn oil, the exposure continue for 28 consecutive days.

Blood sampling procedure

Twenty four hours after the last exposure to chlorpyrifos, drawnwith blood was taken from all animals in this experience by heart puncture put into citrate and heparinized tubes for estimate blood indices, other blood tubes without any anticoagulant that using for biochemical evaluation. The without heparinized tubes were left for about 30 min to allow blood coagulation. Then, the blood centrifuged at 3,000 rpm for 10 min to obtain serum kept in the freezer at -20° C until conducting biochemical assay.

Blood sampling analysis ; number of red blood cells, packed cell volume , hemoglobin, number of leucocytes, erythrocytes indices and platelet number were determined via using standard protocol with the number 60, hemotology analyzer, Genix & USA.

Biochemical evaluation

Measurement the serum malondialdehyde (MDA) by using method of (Ohkawa *etal.*,1979), serum total antioxidant capacity (TAC) was evaluated according method described by (Bartosz ,2003) using ELISA kit , while, inflammatory markers; C-reactive protein (CRP) and Tumor necrosis factor-alpha (TNF-a) were estimated by ELISA using kits according to (Naz *etal.*,1995 and Pradhan *etal.*, 2001).

Histopathological investigation

After 28 days plus 1 week acclimatization, the rabbits were anesthetized and sacrificed, after separated tissue specimens of liver and kidney the specimens were washed with normal saline , then the specimens fixed in 10% neutral formalin solution, the specimens were then dehydrated in sequent ethanol dilution and paraffin- embedded, specimens were sectioned with thickness 5 µM, then slides were staining with hematoxylin-eosin stain to estimate for histopathological changes by light microscope (Suvarna *etal.*,2018).

Statistical Analysis; Data are represented as means ± SE of the mean, Compared between intact animals and experience animals were done by using Student's t-test, a P values ≤ 0.05 indicated statistical significance

Results and Discussion

Pesticides (Organophosphrous) are most widely used in agricultural and domestic applications, the continued exposure to pesticides produced hazard to human and animal health which including many physiological and histopathological alterations (Surat *etal.*,2020). In current study, the observed data in table (1) manifested there is a significant rise of MDA value in concurrently with significantly lower TAC value in group exposed to chlorpyrifos compared to group without exposure, this is an indication of depletion of endogenous antioxidant and increased production of ROS , this reflects greater lipid peroxidation provoked by exposed to insecticide CPF, consequently cellular damage this considered as the major pathway for the CPF toxicity(Lee *etal.*,2017 and A.Jabłonska-Trypu , 2017).The findings in this work are similar with outcomes those described by (Saoudi *etal.*,2018) who demonstrated that increased MDA value in female rats exposed to CPF this could be due to the a decrease in antioxidant enzyme (GPX) activity that removes lipid peroxides and hydroperoxides or as is result to diminished antioxidant enzyme (CAT) activity and glutathione (GSH) levels, this related to

the suppression of their isoenzymes through oxidation of a cysteine. Also (Abdollahi *etal.*,2004; Aly *etal.*,2010 and Afolabi *etal.*,2019) who noticed that low level of TAO resulted from CPF metabolism which stimulates production of ROS thereby increasing the formation of index oxidative (MDA) as is result to increased lipid peroxidation, this process that occurs mainly through mitochondrial destabilization and imbalance of oxidant - antioxidant, hence leading to DNA damage, membrane loss and the death of cell when exposure to insecticides and other environmental pollutants. Also the outcomes recorded in table (1) illustrated that the oral CPF exposure induced markedly elevation in TNF-alpha and CRP values corresponding with health rabbits this May be explained that the metabolism of CPF is positive correlated to production of ROS thereby provoking inflammatory response, the stressful and inflammation are related to a feedback cycle as that free radicals promote the transcription factors which upregulate cytokines expression (Gangemi *etal.*,2016 and Abdel Aziza *etal.*,2021). the data are similarly to those results obtained by (Thandar *et al.*,2021 and Khan *etal*, 2008)who demonstrated that the exposure to insecticides causes inflammatory damage evidenced by increasing values of inflammatory marker CRP and TNF-α. these results also are consistence with reported by(Cauvi *etal.*,2017 and Ruíz-Arias *etal.*,2022) who found that the toxic material stimulates the neutrophil and macrophages and elicits cytokines mediators via provoking and development of the inflammatory response. Furthermore, the oxidative damage is accompanied by inflammation process through triggering the transcription factors of reduction –oxidation-sensitive that controlled the gene expression of cytokines mediators and antioxidant (Astiz *eta*l.,2012).

The results indicated in table (2 ,3) revealed that the animals exposed to chlorpyrifos (CPF) suffering from microcytic hypochromic anemia comparison to intact group evidenced via a marked decline in the values of RBC, erythrocyte indices (MCV, MCH and MCHC), PCV and Hb as comparison to intact group, this type of anemia can be come in part from the impacts of oxidative stress generated by the CPF on RBC(Ruíz-Arias *etal.*,2022). In another study (Amaeze *etal.*,2020) who showed that red blood cells are particularly vulnerable to oxidative damage because they are exposed to greater concentrations of oxygen and, thus, Hb can easily be oxidized. in other point , the exposure to pesticide cause to inhibition of ALAD activity (d-aminolevulinic acid dehydratase, its suppression may be as a result to the connection of pesticide with sulfhydryl group, the inhibition of ALAD enzyme lead to decreased heme biosynthesis consequently result in lower levels of Hb and RBC and eventually anemia (Barathinivas *etal.*,2022). also (Luty *et al.* 2001)reported that the exposure to pesticides resulted in anemia this attributed to oxidative stress in blood cell membranes cause to disturb structure of membrane hence lysis of red blood cells in mice. Beside this, exposure to pesticide induced The hypo-ventilation, decreased in the haemoglobin amount to loading O2, abnormal blood flow, failure of Hb to release bound O2 at tissue sites thereby decreased arterial O2 saturation (Haratym-Maj , 2002). also the findings observed in table (3) indicted that a significant elevation in leucocytes and platelet numbers in animals exposed to CPF as compared to health animals, this attributed to CPF insecticide is toxicant foreign to the body and trigger immune response once introduced into the biological system, one of such responses is increased leucocytes number (Andreadis *etal.*,2013). These findings are agree with result reported by(Ahmed, 2021)who exhibited that the administration of pyrethroid insecticides induced a cytotoxic effect characterized by an increase of leucocyte number in sheep. While the outcomes in this study are disagree with results described by (Waheed *etal.*, 2011 and Riaz and Yousafzai,2017) who observed that the decreased in leucocyte, Hb and PCV values in rabbits following exposure to lambda-cyhalothrin insecticide or a mixture of pyrethroid insecticides. However,t he results in this study are disagreement with some studies have shown that the unaffects

the hemogram and erythrocyte indices in male and female rabbits exposed to another organophosphorus insecticide(Basir *etal.*,2011 and Boumezrag *etal.*,2021).

The histopathological lesions which observed in the renal and hepatic tissues were correlate to response for insecticide CPF exposure, in liver examination revealed cytoplasmic vacuolization and degenerative of hepatocyte, also dilatation and congestion in central vein in fig(2), While the in kidney the renal cortex revealed tubular lesions, including tubular epithelial degenerations, vacuolations and severe dilation of renal tubules after CPF exposure in fig(4) as compared to normal architecture in fig(1and 3), these lesions might be related to the stressful and harmful impacts of the insecticides via the further-production offree radicals and its accumulation as the consequence of increased lipid peroxidation by cyanides and aldehydes causing to injury in the membrane components of the hepatocytes and renal tissue (Bojarski *eta*l.,2018**)**. Lesions in liver and renal are consistent to those lesions described by (Pal *etal.*,2012) who demonstrated that the exposure to CPF result in pathological lesions in architecture of liver and kidney of common carp. Similarly lesions provoked in liver and kidney after exposed to pesticide was reported by (Jama *etal.*,2016l). In present study revealed lesions in liver and kidney corroborating those of (Saoudi *etal.*, 2021,) who reported that CPF, pesticide, induced similar liver and kidney histopathological alteration in rats exposed.

Conclusion: we concluded from findings in this study the oral exposure to CPF evoked inflammation response and oxidative damage as represented by pronounced elevation in levels of CRP,TNF-alpha and MDA biomarkers as well as pathohistological alterations were observably in liver and kidney that indicating that chlorpyrifos elicits numerous adverse effects noteworthy making it a major public health issue.

Table(1) Effect exposure of chlorpyrifos on MDA,TAC, TNFα and CRP values in normal control and experimental rabbits

Groups	MDA µ mol / L	TAC µ mol / L	TNFα ng/ml	CPR mg/L
Normal control corn oil 1ml	0.98 ±0.2b	1.74 ±0.12 a	24.33 ±1.66 b	4.84±0.55b
CPF 33.3mg/kg Dissolved in 1ml corn oil	1.61±0.13a	0. 54±0.046 b	32.47±2.39a	9.32±0.43a

Values are presented as mean ± SE. Values with different small letters are statistically different among study animals at P≤ 0.05

Table(2) Effect exposure of chlorpyrifos on blood indices in normal control and experimental rabbits

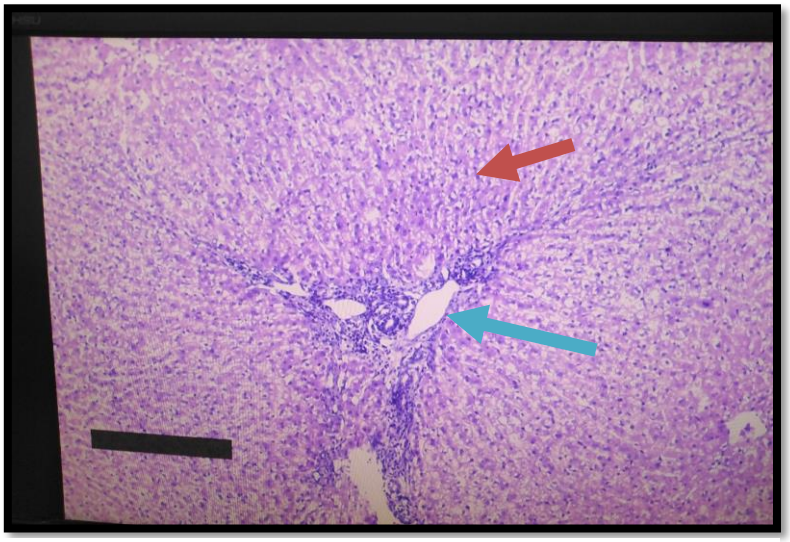
Groups	RBC count X10^6 /µL	PCV%	Hb g/dl	TWBCcount X10^3 /µL
Normal control corn oil 1ml	4.53±0.41 a	45.00±1.07 a	10.95±1.36a	5.65±0.21b
CPF 33.3mg/kg Dissolved in 1ml corn oil	3.16±0.59 b	37.20±1.42b	7.64±0.27b	7.13±0.32 a

Values are presented as mean ± SE. Values with different small letters are statistically different among study animals at P≤ 0.05

Table(3) Effect exposure of chlorpyrifos on erythrocyte indices and thrombocytes in normal control and experimental rabbits

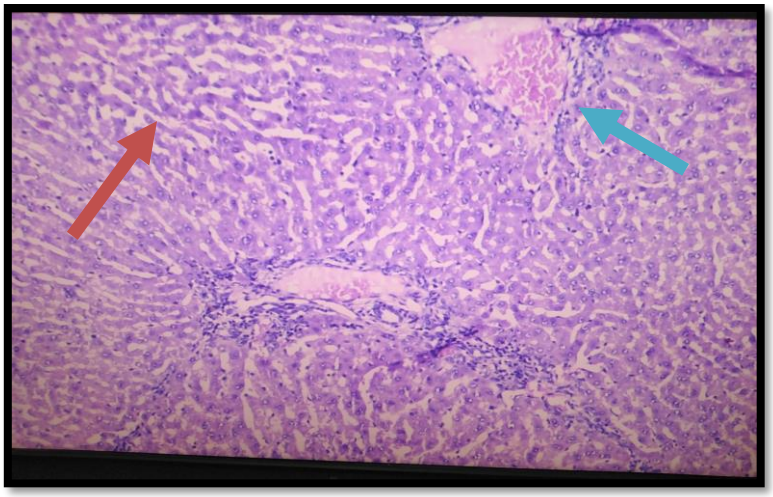
Groups	MCV (fL)	MCH pg/dl	MCHC %	PLT x10^3 /µl
Normal control corn oil 1ml	78.13±2.14 a	27.26± 1.87a	29.68±1.90a	281.53±3.01b
CPF 33.3mg/kg Dissolved in 1ml corn oil	67.04±1.72 b	19.83±1.12b	23.13±2.10b	296.88±2.59a

Values are presented as mean ± SE. Values with different small letters are statistically different among study animals at P≤ 0.05

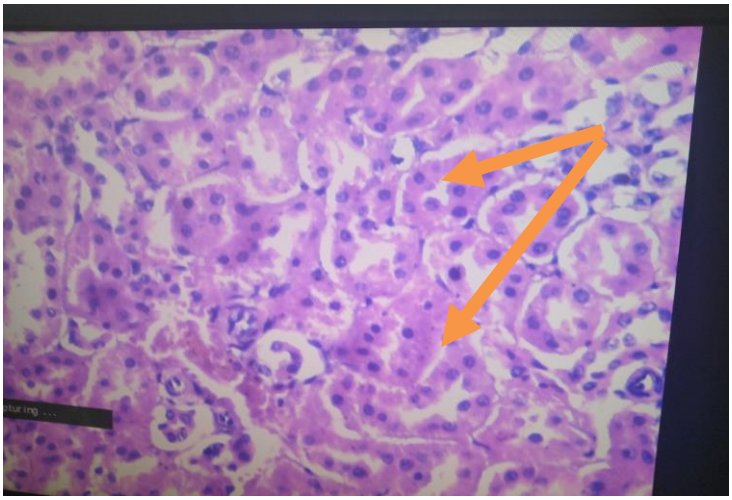


Figure(1):section of liver from health rabbits, showing normal hepatocyes (→)and central vein (→)

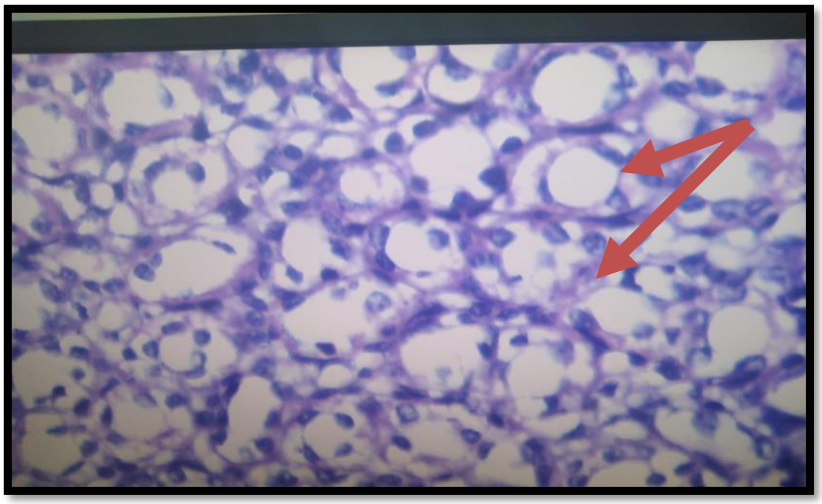
H&E. stain X100



Figure(2): section of liver from rabbits exposed to CPF, showing of degeneration and vacuolation of hepatocyes(→) , minimal dilatation and congestion in central vein(→) H&E. stain x 100



Figure(3):section of renal of health rabbits, showing normal structure of renal tubules( ) H &E. stain x 400



Figure(4): section of renal from rabbits exposed to CPF, showing vacuolation and degenerations tubular epithelial and dilation of renal tubules ( )H&E. stain x 400

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