Chlorfenapyr Induce Oxidative Phosphorylation Deficiency in Exposed Rat and the Quinoa Effective Role

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ABSTRACT

The present study investigates uncouple and pro-oxidant effects of chlorfenapyr (CFp) on rat liver and spleen mitochondria respiratory complexes and evaluates the quinoa seeds (Q) (30% from the basal diet) effects on CFp exposed rats. Repeated sublethal oral doses of CFp (180 mg / Kg body weight; 1/3 LD_{50}) was given to rat. Liver and spleen mitochondria were isolated at the end of the fourth week after dosing. Changes in liver and spleen mitochondrial respiration complexes, and concomitant oxidative stress and antioxidant alterations were examined. Since, CFp provokes a significant reduction in the activity of NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), ubiquinone cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV), superoxide dismutase (SOD), and reduced glutathione (GSH) level. In addition, a significant increase in ATP synthase (complex V) and malondialdehyde (MDA) levels were observed in both liver and spleen mitochondria, indicating clearly an intense peroxidation within mitochondria. So, the results conclude that hepatic and splenic respiratory complexes in rats were severely compromised following CFp exposure. Moreover, Q enhanced diet neutralizes the CFp adverse effect that encourages its use as a mitochondrial enriches source and a protective antioxidant food supplement.

Key words*:* Chlorfenapyr, Electron transport chain, Mitochondria, Oxidative stress, Quinoa.

INTRODUCTION

In the mammalian body, the liver is the major detoxifying organ and is also involved in xenobiotic metabolism. Although the liver, and spleen work together to activate the defense response to blood-borne pathogens through innate and adaptive immunity, the spleen acts as the center of the blood defense system (Kashimura, 2020). In physiological conditions, mitochondria have significant roles in the metabolism and energy regulation as a source of reactive oxygen species (ROS) that generating approximately 85% of the total cellular superoxide radical (O_2•-) (Samarghandian *et al.*, 2016). Mitochondrial impairment can induce oxidative stress and reduce adenosine triphosphate (ATP) content (Samarghandian *et al.*, 2015). The oxidative attack on the mitochondria is also essential in these events. Impairment of mitochondrial function is the primary mechanism involved in the pathogenesis of neurological disorders (Samarghandian *et al.*, 2016). Mitochondria produce (O2•-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH•) as byproducts of molecular oxygen consumption in the electron transport chain (Barbosa *et al.*, 2020). The accumulated O_2• is eliminated by manganese superoxide dismutase (MnSOD), which generates H_2O_2. The mitochondrial glutathione system plays a crucial role in reducing H_2O_2 and protects mitochondria against oxidative stress. Various pesticides can cause impaired energy regulation and cell dysfunction, and finally, cell death has been observed in many neurological disorders (De Castro *et al.*, 2011).

Under conditions of mitochondrial dysfunction, ATP deficiency can lead to failure of the Na+/K+ ATPase, and in primary sensory neurons, this may contribute to the ectopic activity characteristic of neuropathic pain (Lim *et al.*, 2015). The oxidative stress of mitochondrial proteins, lipids, and antioxidant enzymes can be seriously harmful.

Lipid peroxidation (LPO) is caused by free radicals that lead to oxidative destruction of polyunsaturated fatty acids, resulting in toxic and reactive aldehyde metabolites such as malondialdehyde (MDA) (Yaman and Ayhanci, 2021). MDA causes mitochondrial dysfunction by enhancing reactive oxygen species (ROS) generation and modulation of mitochondrial proteins (Caldiroli *et al.*, 2020).

Pesticides are rapidly metabolized in the liver by hydrolytic ester cleavage, and oxidative pathways by cytochrome P450 enzymes produce ROS (He *et al.*, 2020). CFp is a broad-spectrum pyrrole insecticide, which show contact and stomach toxicity (Gunning and Moores, 2002; N'guessan *et al.*, 2007) . It's used to manage veterinary and agricultural pests that have developed resistance to organophosphate, carbamate, and pyrethroid insecticides (Sleem *et al.*, 2019).

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According to WHO (Tomlin, 2000), CFp is classified as a slightly hazardous insecticide, however, it was banned in Europe due to its high persistence in the environment (Van Leeuwen *et al.*, 2004), and is still widely used in Egypt (Sleem *et al.*, 2019). CFp; a pyrrole insecticide works by targeting the oxidative pathways in the insect's mitochondria thus disrupting ATP production (Black *et al.*, 1994; guessan *et al.*, 2007).

The aim of this work was to evaluate the mammalian toxicity of CFp by investigating the activity changes of the respiratory complexes of liver and spleen mitochondria of male albino rats after sub-chronic exposure to chlorfenapyr such as (NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), ubiquinone cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV), and Mg^{2+} ATP (complex V). In addition, oxidative stress biomarkers; MDA, SOD and GSH were studied.

MATERIAL AND METHODS

Chemicals

Chlorfenapyr (Corps Top 24% SC) was obtained from Agrimar Company for Commercial Agencies. Quinoa seeds were purchased from CEDAR, Phoenicia Group Inc. Montreal, H4S 1T2 QC, Canada. All other reagents and chemicals used in this research were obtained from Sigma- Aldrich Chemical Co. (St. Louis, MO, USA).

Animals

Twenty adult male albino rats, weighing 150 -160 g were obtained from the Faculty of Science; Alexandria University. Rats were allowed to acclimatize for 14 days before the initiation of the experiment under laboratory conditions (12 h light / 12 h dark, 22– 26 °C., 40–70% humidity). To facilitate measures of food intake, rats were housed conventionally in individual stainless steel hanging wire-mesh cages, with food and tap water provided *ad libitum.* On arrival, the rats in each experiment were immediately placed in their respective experimental conditions and allowed access to a pre-weighed amount of food so that the first intake measures could be carried out the next day. All procedures involving animals were performed in accordance with Organization for Economic Cooperation and Development, Acute Oral Toxicity Study in Rodents: OECD guideline, 420 (2001).

Experimental design

The animals were divided into equal four groups (5/each group), and orally treated 5 doses / week over a period of 4 weeks as the following: Group C: Rats were served as control and given commercial basal diet. Group QS: Rats were given QS at 30% from the basal diet. Group CFp: Rats were given CFp 180 mg/kg bw which represents 1/3 LD_{50} (Oral LD_{50} for Corps Top (24% SC) as a commercial chlorfenapyr was 544.3 mg/ kg bw; according to Weil's method (Weil, 1952)).

Group CFp + QS: Rats were given CFp 180 mg/kg bw plus QS at 30% from the basal diet. CFp was orally administrated to animals by esophageal intubation. The animal body weights of all groups were recorded weekly. At the end of the experiment (4 weeks), the liver and spleen were quickly removed, weighed individually and the % of relative organ weight was calculated (organ weight/body weight) X 100. The liver and spleen were washed with 0.9% NaCl and frozen until use.

Isolation of rat liver and spleen mitochondria

Rat liver and spleen mitochondria were isolated using a slightly modified protocol (Krause *et al.*, 2005). Tissue was homogenized with 10 volumes (w/v) of 250 mM sucrose, 50 mM Tris–HCL, 5 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.4 buffer using a glass Teflon homogenizer. The homogenate was centrifuged at 800 xg for 10 min at 4 ºC. Then the supernatant was centrifuged at 8,000g for 10 min at 4 ºC, and the pellets were washed twice and suspended in 250 mM sucrose, 0.5 mM PMSF. The isolated mitochondria was frozen as aliquots and stored at -20 ºC.

Determination of protein

The protein content of mitochondrial preparations was measured by the method of Lowry *et al.*, (1951) using bovine serum albumin as a standard.

Assessment of mitochondrial respiratory complexes

1. NADH dehydrogenase (Complex I) activity

NADH dehydrogenase activity was measured according to Galante and Hatefi, (1978), which depends on the oxidation of NADH, as follows: Incubate mitochondrial protein (40 µg /ml) in the mixture containing 40 mM phosphate buffer, pH 7.4, 0.1% sodium cholate, 1.5 mM potassium cyanide and 1.3 mM potassium ferricyanide, as an electron acceptor for 1 min at 30 °C. The reaction was started by adding 0.14 mM NADH, and a decrease of absorption was followed spectrophotometrically at 340 nm for 1-3 minutes. Results expressed as µmol NADH oxidized / min / mg protein. Rotenone-sensitivity activity was monitored using (1µM).

2. Succinate dehydrogenase (Complex II) activity

Succinate dehydrogenase activity was measured according to the method of King, (1967) . one ml assay mixture containing 0.1 M pH 7.4 phosphate buffer,; EDTA, 0.3 mM; sodium succinate, 20 mM; 2,6-Dichlorophenolindophenol (DCIP), 0.053 mM; potassium cyanide, 1mM and an appropriate amount of enzyme (usually 20 µl) to start the reaction. The activity

of succinate dehydrogenase is followed by the decrease of the absorbance at 600 nm, readings were taken at intervals of 30 seconds for 2 minutes, and expressed as, µmoles of succinate oxidized /min/mg protein.

3. Cytochrome C reductase (Complex III) activity

The reduction of cytochrome c was measured spectrophotometrically at 38 ° by the increase in optical density at 550 nm (Green and Ziegler, 1963). The assay system (1ml) consisted of; 50mM phosphate buffer pH 7.4, 0.1 mM EDTA, 1mg/ml cytochrome c, 1.6mM potassium cyanide, 1mM NADH, 10% sodium cholate, and 10 or 20 µl enzyme last addition. Readings were taken every 15 seconds for 2 minutes. The specific activity was expressed as µmoles of reduced cytochrome c /min /mg protein. The extinction coefficient of reduced cytochrome c at 550 nm was $18.5x10^6$ cm^2 /mole.

4. Cytochrome c oxidase (Complex IV) activity

The oxidation of cytochrome c was colorimetrically measured by the decrease in absorbance at 550 nm Green and Ziegler, (1963). The assay system (1ml) containing; 50mM phosphate buffer pH 7.4, 0.1mM EDTA, 1mg/ml cytochrome c, 10% sodium cholate, and 20 µl enzyme as a last addition. Readings were taken every 15 seconds for 2 minutes. The enzyme activity was expressed as moles of oxidized cytochrome c /min /mg protein. The extinction coefficients of oxidized cytochrome c at 550 nm, was 19.6 mM^{-1} cm^{-1} .

5. *Mg^{2+} ATPase (Complex V) activity*

The basic idea of this method is to measure the amount of inorganic phosphate produced from the hydrolytic reaction of ATP by the ATPase. In this method, mitochondria (1 mg protein/ml) were added to a medium containing, 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$ and 5 mM ATP. Then the mixture was incubated for 5 min. at 37°C in a shaking water bath. The reaction was stopped by the addition of 5% trichloroacetic acid (TCA), and then inorganic phosphate (Pi) was determined calorimetrically at 740 nm (Taussky and Shorr, 1953). The activity of ATPase was expressed as µmole Pi/mg protein/min.

Assessment of mitochondrial oxidative stress biomarkers

a) Superoxide Dismutase (SOD) activity

SOD as an enzymatic antioxidant was measured spectrophotometrically at 25 °C by the method of Marklund and Marklund (1974) with some modifications. The assay medium in a total volume of 1.0 ml contains 50 mM Tris – HCl, pH 8.0, and 0.24 mM pyrogallol. The Autoxidation of pyrogallol was monitored at 420 nm for 3 min in the absence and presence of enzyme. At least three concentrations of the enzyme which produced between 30 to 60 % inhibition of pyrogallol autoxidation were used. One unit of enzyme activity is defined as the amount that produced 50% inhibition of pyrogallol autoxidation under the standard assay conditions. Mitochondrial SOD activities were expressed as Units/ mg protein.

b) Reduced Glutathione (GSH) content

GSH level was determined as described by Ellman, (1959). The mitochondrial suspension in 0.1 M phosphate buffer, pH 7.4 was added to an equal volume of 10% TCA. The mixture was allowed to stand for 10 min in ice bath prior to centrifugation at 4000 xg for 10 min. 2.5 ml of Ellman's reagent (39.6 mg of DTNB and 15 mg $NaHCO_3$ in 10 ml 0.1 M phosphate buffer, pH 7.0) was added to 0.5 ml of the supernatant, shaked and then measured at 412 nm against blank. GSH concentration was estimated and obtained from the GSH standard curve as µmole/g tissue.

c) Lipid peroxidation (LPO) level

Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid (TBA) to give a pink-colored complex absorbing at 535 nm Buege and Aust, (1978). Briefly, 0.5 ml of mitochondrial suspension was reacted with 2 ml of TBA reagent containing 0.375 % TBA, 15 % TCA, and 0.25 N HCL. Samples were boiled for 15 min, cooled and centrifuged. The yields supernatants were measured at 535 nm. The LPO level was calculated using the molar extinction coefficient = 155$mM^{-1}cm^{-1}$,and the results were expressed as nmol MDA/mg protein.

Statistical analysis

All results were expressed as mean ± standard error (SE). The data were analyzed using a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. The criterion for statistical significance was set at $p \leq 0.05$. These tests were performed using the computer software CoStat program, version 6.400 (CoHort Software: Tucson, 1998-2008).

RESULTS AND DISCUSSION

RESULTS

Body and Relative organ weights

No mortality or clinical signs of toxicity was founded in any treated animal. The physiological status of control and treated animals was observed as the change in body and organ weights. Data in Table (1) illustrated a significant ($p \leq 0.05$) decrease in the body weight of CFp treated rats by 19.1 % while, the QS-enriched diet enhanced body weight loss by 11.2% compared to control. The relative liver and spleen weights were increased by 48.7% and 48.8 % respectively related to control. While, the QS-enhanced diet alleviated the effect of CFp exposed rats to 21.21 %

and 4.65 % for the liver and spleen, respectively compared to the control (Table 1).

Biochemical effects of chlorfenapyr exposure

The effects of orally dosed CFp (180 mg/kg bw; 1/3 LD_{50}) on the mitochondrial respiratory chain complexes, the enzymatic and non-enzymatic antioxidant defense, and, the protective effects of QS against CFp in the liver and spleen of male rat were presented in Figures 1and2 and Tables 2and3.

NADH dehydrogenase (complex I) activity

Current data showed that the CFp dosing significantly ($p \leq 0.05$) decreased complex I activity of liver and spleen mitochondria by 36.4 % and 33.2%, respectively, while supplementation with QS modified the CPF toxic effect to 22.4% and 8.5% for liver and spleen, respectively related to control (Fig. 1 and 2). As noted, the attenuation of the toxic effect by QS enhanced diet was more promising in the spleen than in the liver.

Succinate dehydrogenase (complex II) activity

Consistently, the CFp intoxicated animals revealed a significant ($p \leq 0.05$) decline in complex II activity of liver and spleen mitochondria by 38.6 % and 26.4%, respectively, while feeding on QS enhanced diet modulates the CFp effect to 3.1 % and 17.1% for liver and spleen mitochondria, respectively compared to control (Fig.1 and2).

Ubiquinone cytochrome* c *oxidoreductase (complex III) activity

The complex III activities of liver and spleen mitochondria were significantly ($p \leq 0.05$) decreased by 40.1 % and 45%, respectively in rats given CFp. However, concomitantly supplementation by QS improved diet markedly mitigated the CFp effect to be 6.5 % and 15 % for liver and spleen mitochondria, respectively related to control (Fig. 1 and 2).

Cytochrome* c *oxidase (complex IV) activity

Obtained results in Fig. 1 and 2 revealed that the complex IV activity of liver and spleen mitochondria were significantly ($p \leq 0.05$) inhibited by 54 % and 36.4 %, respectively, due to CFp treatment, however, concurrently QS enriched diet supplementation noticeably alleviated the CFp harm effect to 17.6 % and 27.3 % for liver and spleen mitochondria, respectively paralleled to control (Fig. 1 and 2).

Mg^{2+} ATPase (Complex V) activity

Contrary, a significant increase in the mitochondrial ATPase activity by 60.6% and 35.3% for rat liver and spleen, respectively were observed after CFp administration. While the CFp administration concomitantly with QS enhanced diet neutralizes the CFp stimulated effect to 35.7% and 17.6% for liver and spleen mitochondria, respectively regarded to control (Fig. 1 and 2).

Table 1. Relative liver and spleen weights percentage and body weight of male rats after 28 days of treatment with chlorfenapyre (180 mg/kg bw), quinoa seed (30% of basal diet) and their combination

Animal Group	Relative liver weight (%)	Relative spleen weight (%)	Gained body weight (gm)
Control	3.63 ± 0.08^{c}	0.43 ± 0.01^{b}	101.7 ± 1.2^{a}
Quinoa seed	3.5 ± 0.21^{c}	0.49 ± 0.03^{b}	95.0 ± 1.1^{b}
Chlorfenapyr	5.4 ± 0.33^{a}	0.64 ± 0.04^{a}	82.3 ± 1.4^{d}
Chlorfenapyr plus Quinoa seed	4.4 ± 0.1^{b}	0.45 ± 0.01^{b}	90.3 ± 0.8^{c}

Values are expressed as means (Five rats) ± standard error (SE).
* Values in a column with the same letters do not statistically differ at $p \leq 0.05$.

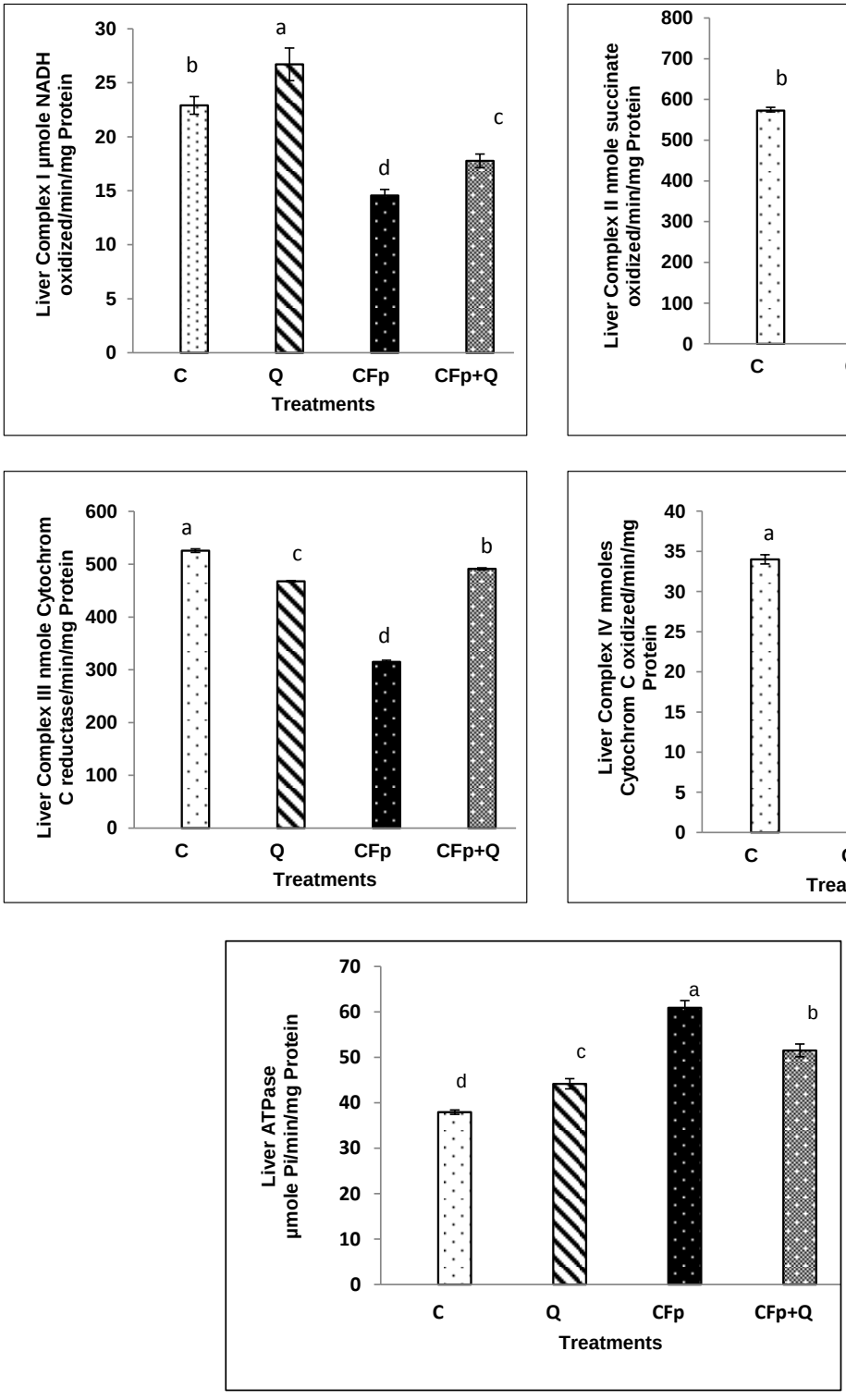

Fig.1. Effects of chlorfenapyr (CFp), quinoa seeds (Q) and their combination (CFp+Q) on the mitochondrial respiratory complexes in rat liver

[a,b,c,d] different letters are significantly different at $p \leq 0.05$

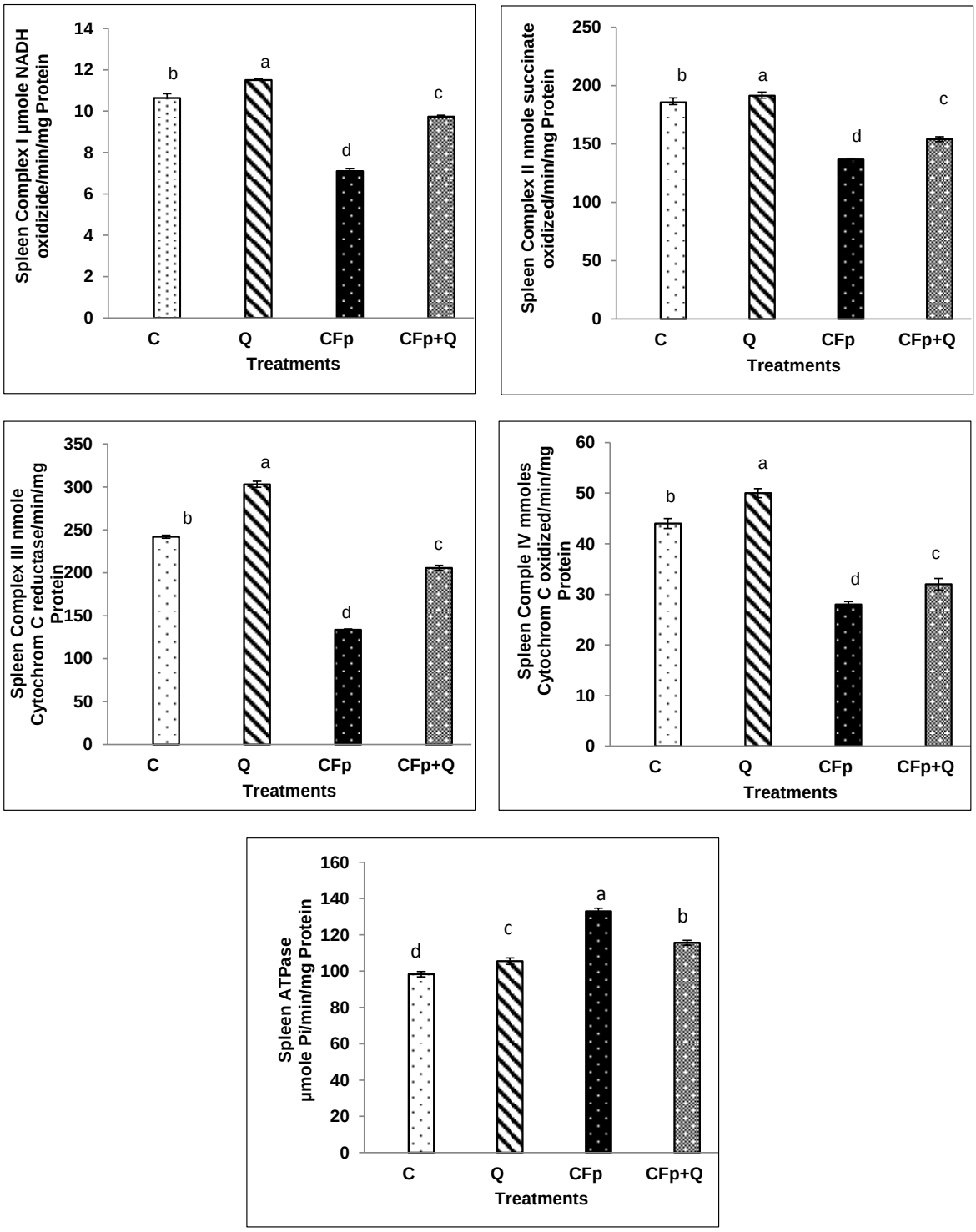

Fig.2. Effects of chlorfenapyr (CFp), quinoa seeds (Q) and their combination (CFp+Q) on the mitochondrial respiratory complexes in rat spleen

a,b,c,d different letters are significantly different at $p \leq 0.05$

Superoxide Dismutase (SOD) activity

The current results in Table (2) showed that SOD activity of liver mitochondria from CFp treated rats was significantly inhibited by 27% however; the QS supplemented diet concomitantly with CFp treatment reduced the damaging effect of CFp to 19.2%. In addition SOD activity of spleen mitochondria was significantly decreased by 32.1% while the QS supplemented diet fed concomitantly with CFp administration attenuated the adverse effect of CFp to 19.6% compared to control (Table 3).

Reduced GSH content

The obtained results showed that CFp treatment reduced the mitochondrial GSH level in the liver by 31.2%, but the QS complemented diet provided concomitantly with CFp administration, mitigated the negative effect of CFp to 16.5% (Table 2). Whereas the mitochondrial GSH level of CFp *in vivo* exposed spleen was declined by 29.8%. However, when a QS supplemented diet was used in conjunction with CFp treatment, the risky effect of CFp was mitigated to 18.8% related to the control group (Table 3).

Lipid peroxidation (LPO) level

Our data showed a significant elevation in liver mitochondria MDA levels due to CFp exposure to be 23%, indicating a loss of structural and functional integrity of the mitochondrial membrane. While QS supplemented diet concomitantly supplied with oral administration of CFp changed the CFp adverse effect to be 3.8% compared to control. In addition, the spleen mitochondria were more affected by CFp treatment than the liver, as the MDA level was raised by 92.6% (Table 2). Whereas, QS enhanced diet given concomitantly with CFp administration attenuated the adverse effect of CFp to 50.5% compared to control (Table 3).

Table 2. Liver mitochondrial oxidative stress biomarkers of male albino rats orally administrated with chlorfenapyr (180 mg/kg bw), quinoa seed (30% of basal diet) and their combination for 28 days (5 doses/week)

Animal Group	Specific Activity of SOD (U/mg protein)	Content of GSH (nmole gluttathion/mg protein)	Content of MDA (nmole/mg protein)
Control	27.78±1.3^{a}	247±1.6^{b}	76.5±0.8^{b}
Quinoa seed	28.18±1.6^{a}	250±1.2^{a}	70.1±1.6^{c}
Chlorfenapyr	20.27±0.9^{b}	170.3±4.0^{d}	94.2±1.3^{a}
Chlorfenapyr plus Quinoa seed	22.43±0.9^{b}	206.3±2.0^{c}	79.4±1.0^{b}

Values are expressed as means (Five rats) ± standard error (SE)

* Values in a column with the same letters do not statistically differ at $p \leq 0.05$.

Table 3. Spleen mitochondrial oxidative stress biomarkers of male albino rats orally administrated with chlorfenapyr (180 mg/kg bw), quinoa seed (30% of basal diet) and their combination for 28 days (5 doses/week)

Animal Group	Specific Activity of SOD (U/mg protein)	Content of GSH (nmole gluttathion/mg protein)	Content of MDA (nmole/mg protein)
Control	19.17±0.4^{b}	240.70±1.2^{b}	22.93±1.6^{c}
Quinoa seed	20.63±0.8^{a}	249.00±1.8^{a}	19.63±0.6^{d}
Chlorfenapyr	13.00±0.6^{c}	169.00±1.5^{d}	44.18±0.9^{a}
Chlorfenapyr plus Quinoa seed	15.40±0.3^{c}	185.30±1.4^{c}	34.52±0.3^{b}

Values are expressed as means (Five rats) ± standard error (SE)

* Values in a column with the same letters do not statistically differ at $p \leq 0.05$.

Discussion

Body and relative organ weights

Exposure to pesticides is associated with the structural damage to organs and tissues along with pathological and inflammatory changes resulting in altered morphology of affected organs and caused severe acute and chronic poisoning (Arfat *et al.*, 2014; El-Gendy *et al.*, 2019). The present study revealed, a significant decrease in the body weight of CFP treated rats which may be attributed to a reduced food intake (anorexia or food avoidance), poor food tastiness or increased degradation of lipids and protein as a result of CFP treatment El-Gendy *et al.*, (2019). Hepatosplenomegaly is an enlarged liver and spleen caused by hepatic disorders (Costa, 2008). As regards the change of organ weight in treating animals was in accordance with those obtained by El-Gendy *et al.*, (2019) who observed a reduction of the body weight and an increase in the relative organs weight of mice or rats exposed to ethoprophos and chlorfenapyr, respectively. Liver enlargement could be induced as a result of; 1) the maintenance of the liver normal functional capacity (Robinson and Yarbrough, 1978), 2) accumulation of fat, mainly triglyceride (Laothong, 2016), a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation , or 3) the increased demands for the detoxification of toxic compounds, Kadota *et al.*, (1976). In contrast; Bhatnagar and Jain (1986) stated that the increase in liver weight is not necessarily considered toxic lesions since this effect is observed in a large number of compounds.

Biochemical findings

The effects of orally dosed CFp (180 mg/kg bw,; 1/3 LD_{50}) on the mitochondrial respiratory chain complexes, lipid peroxidation, the enzymatic and non-enzymatic antioxidant, defense, and, the protective effects of QS against CFp in liver and spleen of male rat were brief in Figs. 1–2 and table 2-3.

Mitochondria play an important role in creating cellular bioenergetics that produces NADH and ATP molecules via oxidative phosphorylation. These functions are linked to neurodevelopment, connections, tissue differentiation, and plasticity (Bergman and Ben-Shachar, 2016). Mitochondrial dysfunction is a fundamental pathogenic mechanism that leads to several significant toxicities in mammals, especially those associated with the liver and spleen (Zanoli *et al.*, 2012). Numerous studies have revealed that mitochondrial dysfunction is a significant mechanism of drug-induced toxicity, as well as the primary mechanism in several disorders and a prominent toxicological target (Bergman and Ben-Shachar, 2016). In our results, CFp caused mitochondrial dysfunction in rat's liver, which was confirmed by inhibiting the mitochondrial NADH dehydrogenase. This finding may depend on the configurationally structure of this enzymatic complex, which is made up of at least 40 different polypeptides firmly embedded in the inner mitochondrial membrane (Küffner *et al.*, 1998). The present findings are consistent with the data of Agrawal *et al.*, (2015) who observed that cypermethrin alters the mitochondrial proteome, reduces the membrane potential, decreases the mitochondrial complex I activity, and increases apoptosis in rat's neurons. In agreement lambda cyhalothrin significantly reduce the NADH dehydrogenase activity in hepatic mitochondria of treated rats (Hamed, (2017). Also, (Guven *et al.*, 2018) reviewed that permethrin and cyhalothrin caused the inhibition of complex I in isolated rat liver mitochondria which may be related to ROS formation. Indeed, the lack of NADH oxidation strongly reduces NAD+ levels, thus impeding the activity of the different NAD+-dependent dehydrogenases of the β-oxidation and TCA cycle pathways (Massart *et al.*, 2013). Impairment of the TCA cycle can lead to hyperlactatemia and lactic acidosis because the conversion of metabolized pyruvate to lactate by lactate dehydrogenase is favored by NADH accumulation (Margolis *et al.*, 2014).

Biochemical and immunohistochemical analyses have demonstrated that mutations in complex II-related genes often result in the reduction or loss of a functional enzyme complex, which may in turn lead to either the accumulation of succinate King *et al.*, (2006) or the generation of reactive oxygen species (ROS), due to frustrating electron transport (Ishii *et al.*, 2005; Slane *et al.*, 2006). Present data showed a decrease in complex II activity of liver and spleen mitochondria as a result of toxic effects of the repeated sublethal dose of CFp. These data are consistent with previous researches that found other pesticides inhibited the activity of complexes I and II in the cortex, cerebellum, and brain stem of acutely monocrotophos-and dichlorvos-treated animals. This inhibition was also seen in rats exposed to dichlorvos and parathion in their liver and brain mitochondria (Karami-Mohajeri and Abdollahi, 2013). In addition, suppression of succinic dehydrogenase activity, which is involved in TCA and complex II, was measured in the rat brain following acute and subacute beta-cyfluthrin exposure (Guven *et al.*, 2018) .

Complex III is a key site of reactive oxygen species (ROS) generation within mitochondria. A dysfunction of its catalytic domain in iron–sulfur

protein blocks electron transport to cytochrome c_1 and subsequently decreases complex III activity in ischemia-damaged mitochondria (Lesnefsky, 2001). The obtained result revealed a significant reduction in liver and spleen mitochondria complex III activity. In line with Karami-Mohajeri and Abdollahi (2013) who reviewed that mevinphos acute exposure resulted in depletion of NADH cytochrome C reductase (Complexes I and III), and succinate cytochrome C reductase (Complexes II and III) in pheochromocytoma cell lines. In contrast, Lee *et al.* (2021) postulated that two chlorinated hydrocarbons compounds; chlordane and β-hexachlorocyclohexane (β-HCH), were associated with increased the specific activity of mitochondrial complexes I and III of exposed zebrafish embryos.

The complex IV is the last electron acceptor of the respiratory chain, involved in the reduction of O_2 to H_2O (Castresana *et al.*, 1994). Our data exhibited a significant inhibition of complex IV activity in liver and spleen mitochondria, which in agreement with other studies of acutely monocrotophos-and dichlorvos-treated rats, that shown a suppression of complexes I, II, and IV activity in the cortex, cerebellum, and brain stem causes organophosphate-induced delayed neuropathy (Masoud *et al.*, 2009). Similar results were obtained by Delgado *et al.* (2006) who concluded that malathion causes oxidative stress which could be due to inactivation of mitochondrial respiratory complexes (II and IV) in central nervous system (CNS) of intoxicated rats.

The enzyme ATP synthase (complex V) is not only responsible for the synthesis of ATP from ADP and inorganic phosphate (Pi), it is also capable of a reversible reaction and can hydrolyze ATP. It's a multi-component structure that spans the inner membrane of mitochondria, the cell's energy generators (Mühleip *et al.*, 2019). In the present study it was found that CFp intoxication stimulated the complex V activity in rat liver and spleen. We can conclude that chlorfenapyr poisoning increased complex V activity by altering mitochondrial membrane permeability and disrupting calcium homeostasis. These results were in agreement with (Delgado-Coello *et al.*, 2006) who postulated that elevated cytoplasmic calcium activates a variety of enzymes with membrane damaging effects and ATPases are among the major enzymes that are involved in calcium activation. In addition, Abdel-Mobdy *et al.* (2019) found that technical and formulated dimethoate stimulated the activities of the respiratory system (Cytochrome-c-oxidase and succinate-cytochrome-c-reductase in brain, liver and kidneys tissues of male albino rats. In contrast to previous findings, Zanoli *et al.* (2012) found that, abamectin has a direct effect on FoF1-ATPase (complex V) and behaves similarly to oligomycin,; a complex V specific inhibitor. In addition, Abdel-Razik, (2019) found that imidacloprid-treated mice had significantly lower brain mitochondrial ATPase activity. Furthermore, the activity of adenosine triphosphatase (complex V) in the brain mitochondria of abamectin-exposed rats was shown to be significantly reduced (Abdel-Razik, and Hamed, 2015). In addition, lambda cyhalothrin intoxication suppressed complex I and V activities in rabbit liver *in vitro* Hamed, (2017) .

Antioxidant enzymes are proteins that catalyze the conversion of reactive oxygen species (ROS) and their metabolites into nontoxic, stable compounds. As a result, it's the most significant barrier against oxidative stress-induced cellular damage. Antioxidant defenses mainly consist of enzymatic as SOD and non-enzymatic as glutathione (Thowfeik, 2016). The current results showed that SOD activity of liver and spleen mitochondria was significantly inhibited. The decreased SOD activity in CFp intoxicated rat may be owed to the consumption of this enzyme in converted O^{2-} to H_2O_2. Wheeler et al., (2001) observed that mitochondrial SOD activity had lowered by half, resulting in a functional decrease in oxidative phosphorylation, an increase in oxidative stress, and an increase in the rate of apoptosis. According to the findings, Mn SOD plays a crucial role in the redox state of mitochondria in cells and tissues (Buettner, (2011). These results are in line with Ranjbar *et al.*, (2010); Wani *et al.*, (2011) who investigated the decrease of mitochondrial function in the rat brain after chronic exposure to malathion and dichlorvos. They further claimed that malathion and dichlorvos cause mitochondrial protein oxidation and disruption of Mn-SOD (mitochondrial SOD) activity, resulting in mitochondrial DNA oxidation and brain damage.

Mitochondrial GSH plays a key role in order to avoid the excessive accumulation of hydrogen peroxide and subsequent oxidative stress (Marí *et al.*, 2013). It is the primary non-protein thiol in cells, with the redox-active thiol of its cysteine moiety serving as a cofactor for a variety of antioxidant and detoxifying enzymes. GSH is synthesized exclusively in the cytosol from its constituent amino acids, yet it is dispersed throughout the body, including mitochondria, where its matrix concentration equals that of the cytosol. This property and its negative charge at physiological pH, suggests the existence of specific carriers that transport GSH from the cytosol to the mitochondrial matrix, where it is involved in the detoxification of lipid hydroperoxides and electrophiles as well as the defense against respiration-induced ROS.

The current research revealed a significant reduction in liver and spleen GSH content which might be due to either GSH synthesis suppression or increased GSH consumption for toxicant induced free radicals

detoxification. The decrease in mitochondrial GSH suggests that CFp oral administration might lead to excessive free radicals production. These free radicals might be attacking the thiol group of cysteine residues and polyunsaturated fatty acids of biological membranes (Raina *et al.*, 2009). These findings run parallel with that previously reported by Sharma *et al.*, (2014) who recorded significant rise in lipid peroxidation and debility in glutathione contents after administration of cypermethrin (at the dose of 3.83 mg / kg bw for 7 days) in Wister rat brain. Furthermore, Abbassy *et al.*, (2014) observed a significant reduction in GSH level after dosing rats with 2.6 mg/kg bw of lambda cyhalothrin for 6 weeks (3doses/week). Likewise, chlordane and β - hexachlorocyclohexane (-HCH) were linked to lower mitochondrial GSH levels in exposed zebrafish embryos, according to Lee *et al.* (2021) study. Reduced GSH levels in mitochondria lead to an increase in H_2O_2 production, which can promote lipid peroxidation, cell membrane damage, and tissue damage (Martín *et al.*, 2000; Şener *et al.*, 2005).

The LPO intensity is one known mechanism of mitochondrial failure in many diseases (Negre-Salvayre *et al.*, 2010). The stimulation of LPO in mitochondrial membranes, combined with oxidative stress, changes their permeability, decreases membrane potential and uncoupled oxidative phosphorylation (Batandier *et al.*, 2004). Our data showed a significant elevation in liver and spleen mitochondria MDA levels due to CFP exposure. Similar results were obtained by Masoud *et al.*, (2009) who found that after sub-acute exposure to monocrotophos or dichlorvos, there was an increase in MDA levels in mitochondria or cytosol as a result of ROS overproduction. Chlorpyrifos also produced mitochondrial oxidative damage by increasing MDA levels inside them (Xu *et al.*, 2017). Previous research has found that lowering GST levels causes an increase in LPO (Dhouib *et al.*, 2014). The present results are consistent with those of Gultekin *et al.* (2001), who linked higher LPO levels to pesticide-induced ROS production and SOD inhibition. Also, Tukhtaev *et al.* (2013) observed that chronic low-dose fipronil exposure elevated LPO in the livers of pregnant rats and their offspring. Furthermore, cypermethrin and methyl parathion significantly increased MDA levels in the hepatic and renal tissues of rats (Gomaa *et al.*, 2011).

In the present study, the organ hypertrophy impact of CFP-exposed rats was mitigated by quinoa seed-enhanced diet, and that may be associated to the quinoa hypolipidemic ability. Two studies showed that polysaccharide fractions purified from quinoa exhibited antioxidant and immunomodulatory activities, evidenced by significant radical scavenging activity and anti-inflammatory effect (Yao *et al.*, 2014; Hu *et al.*, 2017). Another research found that a purified quinoa polysaccharide, SQWP-2, inhibited adipocyte differentiation, so it might be useful as an antiobesity agent (Teng *et al.*, 2020).

In mitochondria, docosahexaenoic acid (DHA) helps reduce oxidative stress by regulating antioxidant signaling pathways (Oppedisano *et al.*, 2020). Six to eight percent of the total fat content of quinoa is α-linoenic acid, an essential precursor of DHA (Gómez *et al.*, 2021). Multiple assays have proven that the antioxidant ability of the active ingredients in quinoa is retained. The results of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS+), oxygen radical absorbance capacity (ORAC), lipoxy genase/ 4-nitroso-N,N-dimethylaniline (LOX/RNO), Trolox equivalent antioxidant capacity (TEAC), cellular antioxidant activity (CAA), ferric reducing sntioxidant power (FRAP), (NO•), NO radical and H_2O_2 scavenging assays all indicate that quinoa presents high antioxidant activity (Ayyash *et al.*, 2018; Balakrishnan and Schneider, 2020; Mufari *et al.*, 2021).

Animal experiments have shown that the consumption of quinoa increases the activity of antioxidant enzymes, including SOD, glutathione peroxidase (GPx) , and catalase (CAT); as well as affected antioxidant- biomarker that reduces LPO, as evidenced by a decrease of MDA and an increase GSH levels (Ali, 2019; Mohamed *et al.*, 2019; Ahmed *et al.*, 2020; Al-Qabba *et al.*, 2020; Abdel-Wahhab *et al.*, 2021), this is consistent with our results.

Quinoa's antioxidant capacity may owe to the presence of phenolic components, such as flavonoids (Ahmed et al., 2020; Paśko et al., 2009). Balakrishnan and Schneider, (2020) found that following in *vitro* digestion, seven out of eleven flavonoids remained intact. It means that digestion will have no effect on the function of these flavonoids, which is in line with Chirinos *et al.* (2020) finding's. Balakrishnan and Schneider, (2020) also observed that gastrointestinal digestion of quinoa flavonoids resulted in a twofold increase in antioxidant capacity, implying that direct quinoa eating may be sufficient to provide a good antioxidant impact.

CONCLUSIONS

The current study found that sublethal oral administration of chlorfenapyr at 1/3 LD_{50} for 28 days, caused cytotoxic changes in the hepatic and splenic mitochondrial respiratory complexes, enzymatic and non-enzymatic antioxidants, thereby weakening the mitochondrial ability of the liver and spleen to manage the CFp oxidative stress. This suggests an overall perturbation of the electron transfer pattern causes mitochondrial dysfunction, abnormalities in

metabolism, and immunity innate in rats. On the other hand, the sufficient dietary intake of QS riches the mitochondria and may provide significant protection against the CFp negative impact.

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الملخص العربي

كلورفينابير يحث خلل الفسفرة التأكسدية في الجرذان المعرضة والدورالفعال للكينوا

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تبحث هذه الدراسة في تأثيرات مركب الكلورفينابير (CFp)على إعاقة إزدواج مكونات الفسفرة التأكسدية ومدى حث المركب لعملية الأكسدة لمعقدات الميتوكوندريا التنفسية في كبد وطحال الجرذان.، وتقييم تأثير بذورالكينوا (٣٠٪ من النظام الغذائي الأساسي) على الفئران المعرضة لـ CFp. أعطيت الجرذان جرعات تحت مميتة من CFp عن طريق الفم (180 مجم / كجم من وزن الجسم والتى تمثل ثلث الجرعة المسببة لموت نصف عدد الأفراد المعاملة). عزلت ميتوكوندريا الكبد والطحال في نهاية الأسبوع الرابع بعد المعاملة، ثم فحصت التغيرات في معقدات تنفس ميتوكوندريا الكبد والطحال ، وما يصاحب ذلك من متغيرات فى مؤشرات الإجهاد التأكسدي. أظهرت النتائج ان المبيد المستخدم يؤدي إلى انخفاض كبير في نشاط كل من: الناد ديهيدروجينيز NADH (المعقد الأول)، السكسينات ديهيدروجينيز (المعقد الثانى)، و يوبيكوينون سيتوكروم سى أوكسيدوريدكتاز (المعقد الثالث)، السيتوكروم سى أوكسيداز (المعقد الرابع)، السوبر اوكسيد ديسميوتيز، وتقليله مستوى الجلوتاثيون المختزل (GSH)، بالإضافة إلى زيادة كبيرة في مستويات الأدينوزين ثلاثى الفوسفاتيز ATPase (المعقد الخامس) ومستوى اكسدة الدهون في كل من ميتوكوندريا الكبد والطحال، مما يشير إلى وجود أكسدة شديد داخل الميتوكوندريا. خلصت النتائج إلى أن معقدات الجهاز التنفسي الكبدية والطحالية في الجرذان تعرضت لخطر شديد بعد المعاملة بالمبيد. علاوة على ذلك فقد لوحظ أن النظام الغذائي المعزز ببذور الكينوا يحيد التأثير الضار للكلورفينابير مما يشجع استخدامه كمصدر لإثراء الميتوكوندريا ومكمل غذائي مضاد للأكسدة.

الكلمات الدالة: كلورفينابير، سلسلة نقل الإلكترون، الميتوكوندريا، الإجهاد التأكسدي، الكينوا