Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice

Slimen Selmi*, Kais Rtibi, Dhekra Grami, Hichem Sebai, Lamjed Marzouki

Laboratory Functional Physiology and Bio-resources Valorisation, Higher Institute of Biotechnology of Beja, University of Jendouba, Avenue Habib Bourguiba, BP, 382, 9000 Beja, Tunisia

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ABSTRACT

The present study was undertaken to determine the effects of malathion exposure on oxidative stress, functional and metabolic parameters in kidney and liver of prepubertal male mice. For this reason, two separated groups of prepubertal male mice were used in this experiment. Animals were divided into two groups, group 1 served as a control and received the corn oil and group 2 was treated with 200 mg/kg body weight (b.w.) of malathion for 30 days. In result, we found that the malathion administration led to the perturbation of biochemical markers and histopathological as well as molecular damages. These changes were accompanied by an oxidative alternation which was evaluated by lipoperoxidation process and MDA production, a diminution of sulfhydril groups (−SH) content and an antioxidant enzyme activities depletion such as total superoxide dismutase (SOD) and its isoforms, catalase (CAT) and glutathione peroxidase (GPx) in both kidney and liver tissues. These changes were related with many histopathological lesions in the liver and kidney tissues. More importantly, this insecticide clearly caused a decline in the GPx-4 expression in liver as well as GPx-3 in kidney. These data suggest that prepubertal male mice exposure to malathion showed a marked deregulation of liver and kidney functions.

1. Introduction

Extensively using of organophosphorus pesticides in different fields such as agriculture, medicine and industry can cause many disturbances in human and wildlife. These organophosphorus (OP) compounds are immediately degraded in the environment. Their concept was introduced following the ban on organochlorines which can bioaccumulate and biomagnify, which results in ecotoxicological effects [1,2]. Particularly, malathion [O,O-dimethyl-S-(1,2-dicarcethoxyethyl) phosphorodithioate] is an OP pesticide habitually used to eradicate ectoparasites, household insects, to conserve stored grain and to eliminate disease-inducing arthropods [3,4,5]. On the negative side, it is one of OPs agents that exerts diverse toxic effects throught the inactivation of serine esterases [6], mostly acetylcholinesterase (AChE) and butyrylcholinesterase which leads to an overstimulation of the cholinergic pathways [7,8]. The OPs can achieve all the tissues leading eventually to several pathological difficulties, this is due to their lipophilic nature and their simple and rapid intestinal assimilation, including a insufficiency of the immune system [9,10] pancreatitis [11], liver disease [12,13] hematological pathosis disorder [14], kidney injury [15],

decrease fertility and reproduction capability [16]. Many studies have reported toxic effects of this OPs in both humans [17,18] and animals [19,20]. Being the main actors of xenobiotic biotransformation, regulation of hepatic gene expression may play a central role in the adaptive response to altered metabolism by changing the capacity of enzymes in relevant metabolic pathways [21]. Hence, liver is the principal metabolizing site for mediating biotransformation of thiono-organophosphates and with kidney contributing to the elimination of toxic products [22]. These tissues are considered among the main targets of malathion toxicity which is mediated through oxidative stress generated by reactive oxygen species (ROS) [23,24,25]. ROS such as superoxide anion, peroxyl radicals, hydroperoxyl radical, hydrogen peroxide are produced from the molecular oxygen as a consequence of normal cellular metabolism [26]. At low or moderate concentrations, ROS are considered as part of normal oxidative metabolism, but at elevated concentrations, they cause tissue injuries, including lipids, proteins oxidation, DNA damage [27], and enzyme inactivation. They are also implicated in many pathological conditions such as cancer, diabetes, cardiovascular, pulmonary and autoimmune diseases, neurological disorders and aging, among others. The main objective of this

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study was therefore to highlight the role of oxidative stress as a precursor of molecular and histopathological complications following subacute exposure of prepubescent mice to malathion.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide, eosin stain, 5.5′-dithiobis 2-nitrobenzoic acid (DTNB), Malathion (98% purity) (fyfanon 50 EC 500 g/l), RPMI (Roswell Park Memorial Institute) and triton X-100 were purchased from SIGMA and Invitrogen.

2.2. Animals and experimental fields

Female and male mice were purchased from Pasteur Institute of Tunis. All experiments were performed according with the local ethics committee of Tunis University for the use and care of animals in accordance with the NIH recommendations. The animals were provided with food (standard pellet diet- Badr Utique-TN) and water *ad libitum* and maintained in animal house at controlled conditions: temperature (22 $\pm$ 2 °C) and 12 h light-dark cycle. Primiparous females were placed three per cage with one male breeder and vaginal smear was examined daily in the evening. At the weaning age (21days), after the lactational period of their offspring (prepubertal male mice) were separated and then randomly divided into two groups of 16 animals each: Group 1 was served as control and received the corn oil. Group 2 received by intragastrique gavage, the malathion in corn oil at the dose of 200 mg/kg, b.w. during 30 days. The age of animals and the used malathion-dose as well as the treatment duration were chosen based on previous work [16].

On the last day of experiment, animals were anaesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) and sacrificed by decapitation. The blood was collected in heparinized tubes and the plasma was obtained after centrifugation at 3000*g* for 15 min. The tissue specimens were removed and placed in a phosphate buffered saline (PBS) solution, homogenized and centrifuged for 15 min at 9000*g*. Organs supernatants and plasma were stored at -80 °C for biochemical parameters determination.

2.3. Evaluation of body organ weights

The initial and final body weights were recorded. Mice in each group were euthanized and their organs were stripped from fatty tissues and blood vessels. Then, these organs were blotted, and their absolute weights were measured. Clinical signs of body and organs were evaluated for toxicological criteria and organ weights were expressed per 100 g body weight to normalize the data for statistical analysis.

2.4. Functional and metabolic parameters determination

To assess the liver function disorders, plasma alanine aminotransferase (ALT), phosphatase alkaline (PAL), aspartate aminotransferase (AST), total and direct bilirubin were measured using commercially available diagnostic kits (Biomaghreb, Ariana, Tunisia).

Concerning renal function damages, plasma urea, creatinine, uric acid and albumin analyses were also performed using commercially available diagnostic kits (Biomaghreb, Ariana, Tunisia).

2.5. Oxidative deterioration of lipids and protein thiol groups determination

Final products of lipiperoxidation, malondialdehyde (MDA) were determined using the method of Buege and Aust [28] and total thiol groups (−SH) concentration was performed according to Hu and Dillard [29].

2.6. Antioxidant activities determination

The activity of superoxide dismutase (SOD) was determined by using modified epinephrine assays and characterization of SOD isoforms was performed using KCN (2 mM), which inhibits Cu/Zn-SOD or H_2O_2 (5 mM), affecting both Cu/Zn-SOD and Fe-SOD whereas Mn-SOD was insensitive to both inhibitor [30,31].

The activity of CAT was assessed by measuring the initial rate of H_2O_2 disappearance at 240 nm [32] and GPx activity was quantified by the procedure of Flohé and Günzler [33].

2.7. Total RNA isolation and RT-PCR analysis

Total RNA was prepared using Trizol reagent according to the manufacturer's instructions. Total RNA (1 µg) reverse was transcribed using MMLV reverse transcriptase (Invitrogen, Tunis, Tunisia) by incubation at 25 °C for 10 min, at 42 °C for 60 min and at 99 °C for 5 min. The synthesized cDNA was amplified using Taq DNA polymerase (Invitrogen, Tunis, Tunisia) and the following specific primers:

GPx-4: **F: 5′-AGTACAGGGGTTTCGTGTGC-3′**
R: 5′-CGGCAGGTCCTTCTCTATCA-3′
GAPDH: **F: 5′-GTGGATATTGTTGCCATCA-3′**,
R: 5′-ACTCATACAGCACCTCAG-3′.

PCR conditions were 30 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, followed by 5 min incubation at 72 °C. PCR products were run on 1.5% agarose gel and then stained with ethidium bromide.

2.8. Histopathological examination

Immediately after the euthanasia, small pieces of both tissues were harvested and washed with ice cold saline, fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. These pieces were cut into 5 µm thick, deparaffinized, hydrated and stained sections with hematoxylin and eosin (HE). The liver and kidney sections were examined in control and malathion treatment.

2.9. Protein determination

Protein concentration was determined according to Bradford method using bovine serum albumin (BSA) as standard [34].

2.10. Statistical analysis

Statistical significance was determined by one-way ANOVA using Statview statistical software. Results were expressed as means $\pm$ standard error of the mean (S.E.M.). The data are repre-sentative of 16 independent experiments. All statistical tests were two-tailed, and a *p* value of 0.05 or less was considered significant.

3. Results

3.1. Body weight, liver and kidney relative weights

As shown in Table 1, the sub-acute exposure of prepubertal male mice to malathion reduced the body weight and mass gain of prepubertal male mice. In contrast, a significant increase in the relative weights of both liver and kidney was observed in malathion-treated mice.

3.2. Liver and kidney functions

According to Table 2, the malathion (200 mg/kg) exposure was associated with liver and kidney dysfunctions in prepubertal male mice, while, a significant increase in some liver biochemical parameters including ALT, AST, PAL and LDH was observed in malathion-treated

Table 1

Body weight, mass gain changes in relative and absolute weights of liver and kidney after sub-acute exposure of prepubertal male mice to malathion.

	CTR	Malathion
Initial body Weight (g)	8,71 ± 0,15	9,33 ± 0,12
Final body Weight (g)	29,76 ± 0,99	21,41 ± 0,57 **
Mass Gain (g)	21,05 ± 0,86	12,08 ± 4,45 *
Absolute Weight of Liver (g)	2,09 ± 0,12	1,67 ± 0,15 *
Relative Weight of Liver (g/100 g PC)	7,03 ± 0,10	7,8 ± 0,09
Absolute Weight of Kidney (g)	0,31 ± 0,01	0,29 ± 0,01
Relative Weight of Kidney (g/100 g P.C.)	1,04 ± 0,01	1,35 ± 0,01 *

Values are means + S.D. of 16 mice in each group.

*and ** represent the statistical difference between control and treated groups, respectively, at $p < 0.05$ and $p < 0.01$.

CTR: control group.

Table 2

Changes in liver function after sub-acute exposure of prepubertal male mice to malathion.

	CTR	Malathion
ALT (UI/l)	42,4 ± 1,5	59,2 ± 2,63**
AST (UI/l)	130,6 ± 2,2	198,8 ± 14,9**
PAL (UI/l)	75,62 ± 11,3	142,6 ± 28,13*
LDH (UI/L)	213 ± 35,6	352,1 ± 8,81**
Bilirubin (mg/dl)	5,10 ± 0,21	6,89 ± 0,31*
total Protein (g/100 g)	10,2 ± 0,51	7,48 ± 0,44**

Values are means + S.D. of 16 mice in each group.

*and ** represent the statistical difference between control and treated groups, respectively, at $p < 0.05$ and $p < 0.01$.

CTR: control group.

mice. Malathion treatment was also associated with hepatotoxicity in male mice as revealed by an increase in direct and total bilirubin.

In kidney, a significant ($p < 0.05$) increase in plasmatic creatinine and urea levels as well as a decrease in albumin and uric acid contents in plasma were observed in mices treated with malathion (Table 3).

3.3. Oxidative stress status

Data from Figs. 1 and 3 clearly revealed that malathion exposure increased significantly ($p < 0.05$) the MDA and H_2O_2 contents, respectively. In contrast, it significantly decreased the thiol ($-SH$) groups (Fig. 2) and reduced the CAT (Fig. 4) and GPx (Fig. 7) activities in both liver and kidney tissues. Total SOD, Cu/Zn–SOD and Mn–SOD activities in liver (Fig. 5) were significantly ($p < 0.05$) reduced in response to malathion exposure. In kidney tissues, total-SOD and Mn–SOD were reduced in malathion-treated mice (Fig. 6).

3.4. Expression of GPx-3 and GPx-4 in liver and kidney

Data from Figs. 8 and 9 showed that liver and kidney express both GPx-4 and GPx-3 isozymes, and that their expressions were remarkably

Table 3

Changes in liver function after sub-acute exposure of prepubertal male mice to malathion.

	CTR	Malathion
Creatinine (μmoles/L)	102,83 ± 5,36	142 ± 6,59**
Uric Acide (μmoles/L)	288,64 ± 14,87	143,17 ± 11,42**
Urea (mmoles/L)	7,06 ± 0,4	9,72 ± 0,32**
Albumin (g/dl)	5,46 ± 0,29	3,62 ± 0,36*
total Protein (g/100 g)	2,98 ± 0,29	3,1 ± 0,15

Values are means + S.D. of 16 mice in each group.

*and ** represent the statistical difference between control and treated groups, respectively, at $p < 0.05$ and $p < 0.01$.
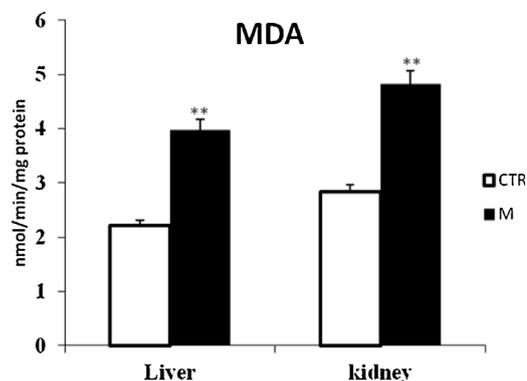
CTR: control group.

Fig. 1. Effects of malathion exposure (200 mg kg^{-1} b.w., p.o.) of prepubertal male mice during 30 days on MDA level in liver and kidney tissues (n = 12). *: $p < 0.05$, **: $p < 0.01$ versus control group.

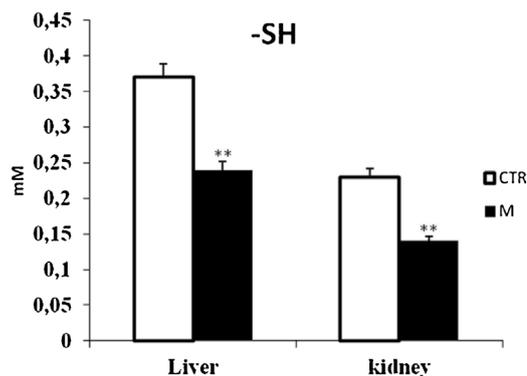**Fig. 2.** Effects of malathion exposure (200 mg kg^{-1} b.w., p.o.) of prepubertal male mice during 30 days on thiols group level in liver and kidney tissues (n = 12). *: $p < 0.05$, **: $p < 0.01$ versus control group.
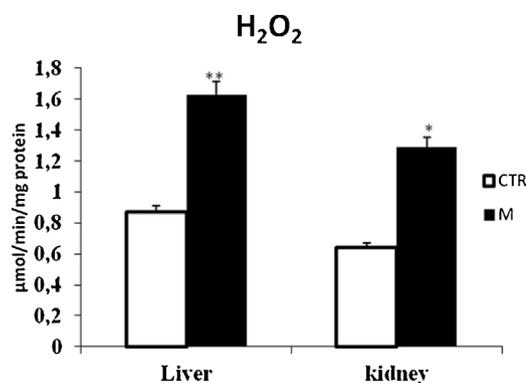

Fig. 3. Effects of malathion exposure (200 mg kg^{-1} b.w., p.o.) of prepubertal male mice during 30 days on H_2O_2 level in liver and kidney tissues (n = 12). *: $p < 0.05$, **: $p < 0.01$ versus control group.

depleted by malathion exposure. In contrast, the GADPH expression remained unchanged in both treated and non-treated mice.

3.5. Histopathological changes in liver and kidney tissues

Light microscopic observation of liver of control mice showed regular and compact configuration with well organized hepatic cell and central vain (Fig. 10A and C). The section of malathion treated mice showed different histopathological alteration. The slide from treatment with malathion showed severe damage in hepatic tissue including prominent enlargement of sinusoids, infiltration of mononuclear cell, dilation, hemorrhage and necrosis (Fig. 10B and D).

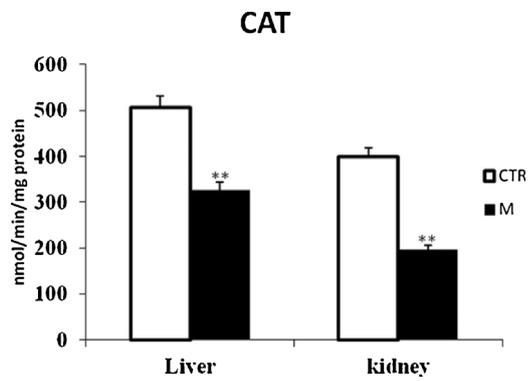Histopathological study on the kidney of control mice showed

Fig. 4. Effects of malathion exposure (200 mg kg⁻¹ b.w., p.o.) of prepubertal male mice during 30 days on catalase activity in liver and kidney tissues (n = 12). *: p < 0.05, **: p < 0.01 versus control group.
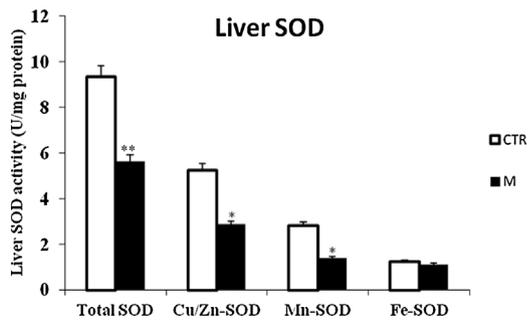

Fig. 5. Effects of malathion exposure (200 mg kg⁻¹ b.w., p.o.) of prepubertal male mice during 30 days on SOD and its isoformes activities in liver tissue (n = 12). *: p < 0.05, **: p < 0.01 versus control group.
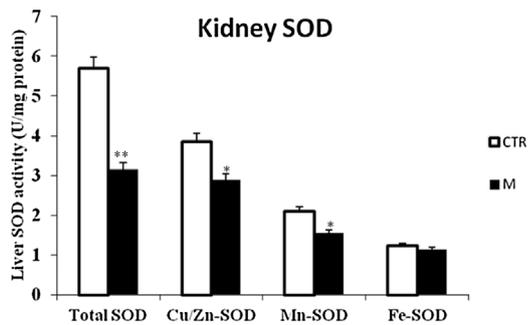

Fig. 6. Effects of malathion exposure (200 mg kg⁻¹ b.w., p.o.) of prepubertal male mice during 30 days on SOD and its isoformes activities in kidney tissue (n = 12). *: p < 0.05, **: p < 0.01 versus control group.
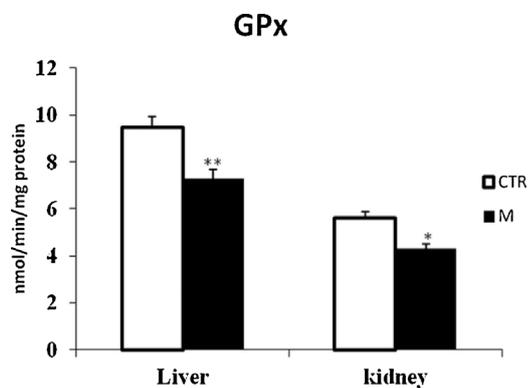

Fig. 7. Effects of malathion exposure (200 mg kg⁻¹ b.w., p.o.) of prepubertal male mice during 30 days on GPx activity in liver and kidney tissues (n = 12). *: p < 0.05, **: p < 0.01 versus control group.
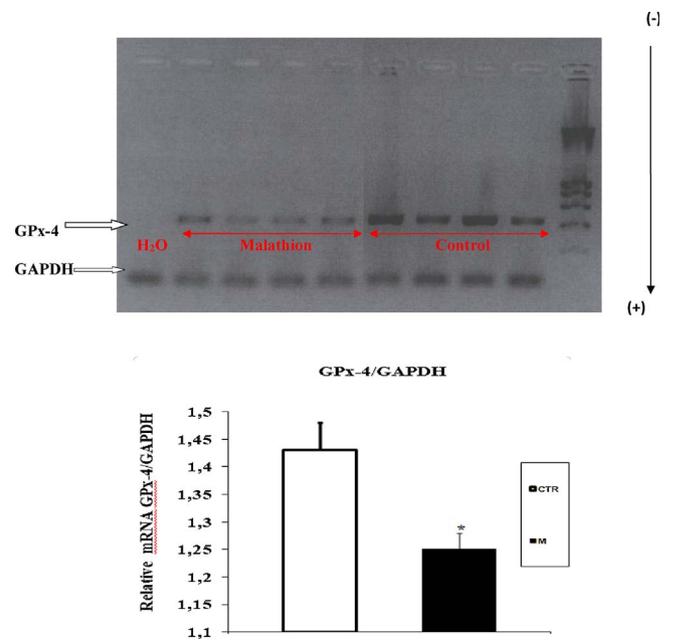

Fig. 8. RT-PCR analysis of malathion effect on GPx-4 expression in liver of male mice. Prepubertal male mice were treated with malathion (200 mg kg⁻¹ b.w., p.o.) during 30 days. The relative expression of GPx isoforms was quantified by densitometry and normalized to GAPDH expression. Data are expressed as mean SEM (n = 4). *: p < 0.05, **: p < 0.01 versus control group.
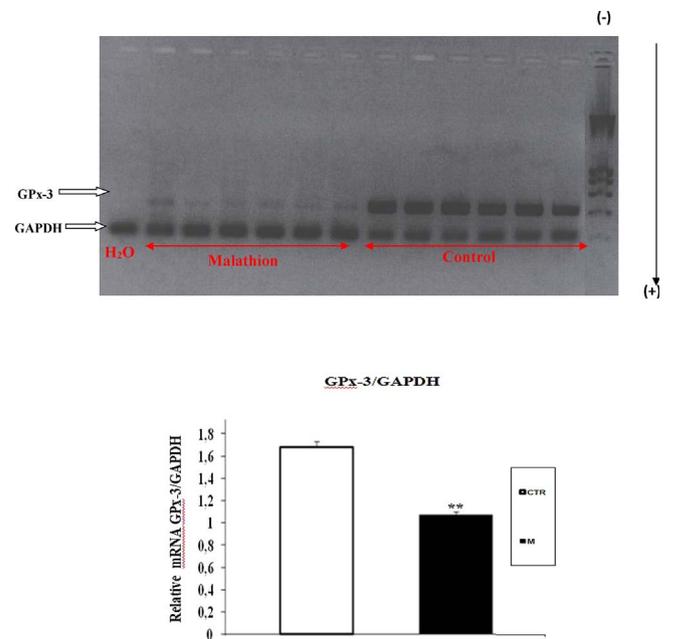

Fig. 9. RT-PCR analysis of malathion effect on GPx-3 expression in kidney of prepubertal male mice. Prepubertal male mice were treated with malathion (200 mg kg⁻¹ b.w., p.o.) during 30 days. The relative expression of GPx isoforms was quantified by densitometry and normalized to GAPDH expression. Data are expressed as mean SEM (n = 6). *: p < 0.05, **: p < 0.01 versus control group.

regular structure with capillaries, tubules, glomerulus, and Bowman's capsule (Fig. 11A and C). On the other hand, the areas of renal cortex containing renal corpuscles and associated tubules expressed more pronounced changes in malathion-treated animals compared with control. In the case of malathion-treated group, highly degeneration of glomeruli, Bowman's capsules and associated tubules structure, shrinkage of glomeruli and edema of renal tubules and raising of urinary space was also noticed (Fig. 11B and D).
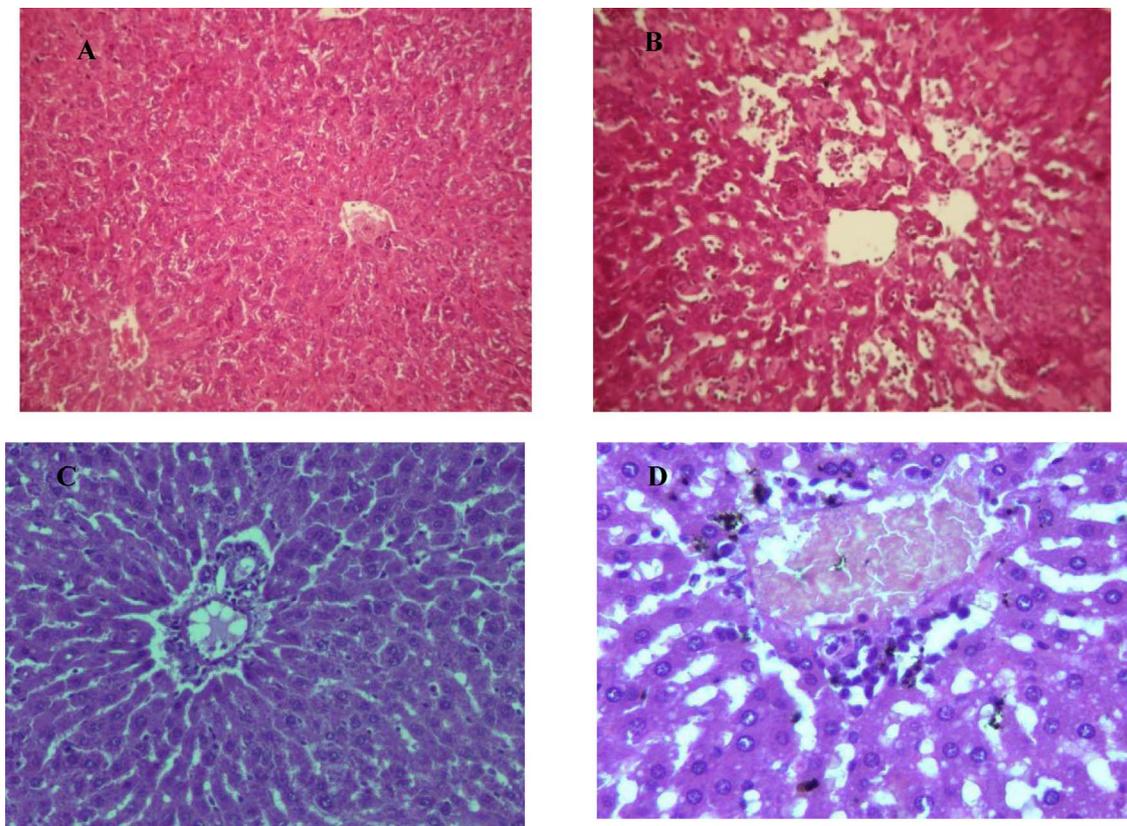
Fig. 10. Liver histology showing the effect of malathion exposure (200 mg kg^{-1} b.w., p.o.) during 30 days of prepubertal male mice. Normal architecture in control group (A) (x100) and (C) (x400). Histological changes in malathion treated group (B) (x100) and (D) (x400).
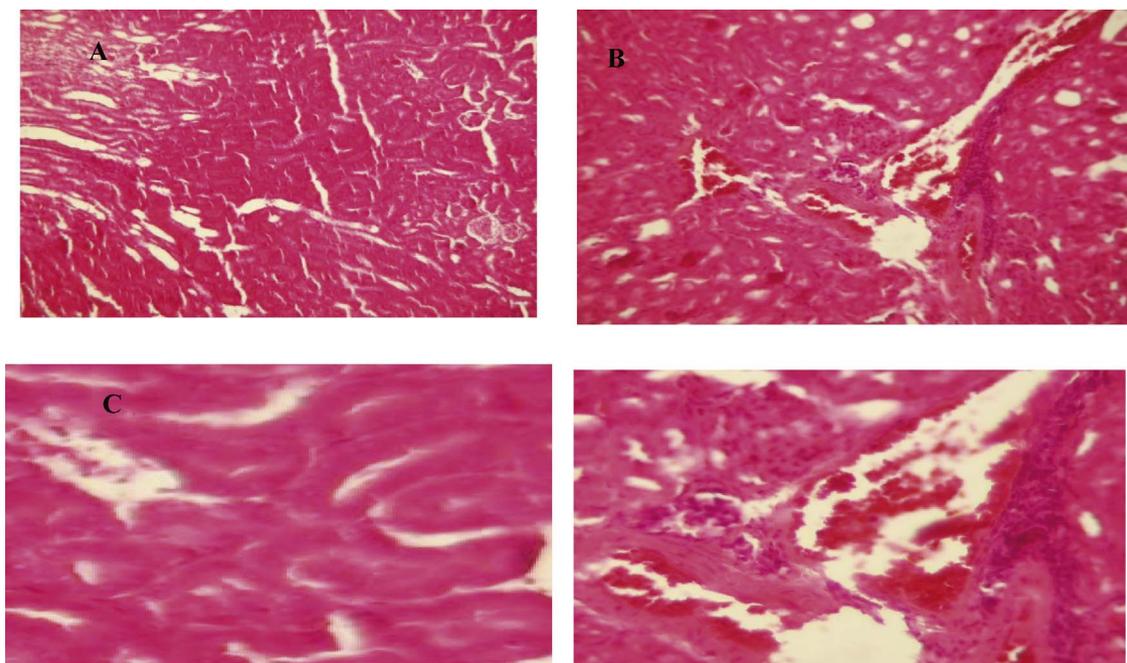
Fig. 11. Kidney histology showing the effect of malathion exposure (200 mg kg^{-1} b.w., p.o.) during 30 days of prepubertal male mice. Normal architecture in control group (A) (x100) and (C) (x400). Histological changes in malathion treated group (B) (x100) and (D) (x400).

4. Discussion

The present research was to evaluate the putative implication of oxidative stress in the sub-acute effects of malathion on the liver and kidney function in male mice. Firstly, we have shown that malathion-induced a significant ($p < 0.05$) reduction of body weight gain.

Conversely, a significant increase of liver and kidney relative weights in mice was observed. These finding are in agreement with previous studies who demonstrated morphologic and symptomatic modifications in morphometric parameters following exposure to malathion. These changes were characteristic of acetylcholinesterase inhibition including accumulation of acetylcholine and subsequent activation of cholinergic,

muscarinic and nicotinic receptors as well as the neurological deficits in male mice exposed to malathion [35]. All these disturbances can lead to various toxic effects in male mice, including their feeding ability, and therefore, their metabolisms performances [36]. The sub-acute administration of malathion lead to an increase in weights of liver and kidney which was associated to remarkable injuries. These damages were assessed by numerous perturbations in the metabolism of these organs. Indeed, increased AST, ALT and ALP activities and depletion in direct and total bilirubin demonstrated a hepatotoxicity effect. In addition, an increase in creatinine and urea levels indicated a kidney dysfunction. These disruptions were observed with several cases of OPs poisoning in rats such as chlorfenvinfos, fenthion and dimethoate [37].

To explain these metabolic perturbations, the transaminases such ALT and AST are major cytolysis markers in the liver and their activities increasing in the plasma of male mice resulted from the impairment and necrosis of the function of tissues with subsequent liberation of enzymes into the circulation from the damaged tissues [38,39]. ALP, which is an important critical enzyme in biological processes, is responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in this enzyme leads to biochemical alternation and impairment in the tissue and cellular function [40]. In addition, it has been reported that the increase in the activity of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis [41].

To explain renal disturbances, these results corroborated with previous studies in adult rats and their suckling pups intoxicated with dimethoate [42] and in adult rats treated with chlorfenvinfos or with phosphorodithioate [43,44]. Furthermore, plasmatic uric acid levels can be influenced by many drugs witch could affect the net reabsorption of uric acid in the proximal tubule of the nephron [45]. Paradoxically, Bosco et al. [46] showed no changes in glomerular filtration rate of *Octodon degus* exposed to malathion (200 ppm) as sole drinking fluid for 90 days. This discrepancy might result from the difference in the sensitivity of two species (O. degus and rats). On the another hand, mercuric chloride and dimethoate lead to an increase in urine volume in adult rats exposed to moderate doses of these compounds [42].

In addition to these manifestations, increased lipoperoxidation assessed in term of MDA, and hydrogen peroxide, decreased thiol groups level as well as a depletion of antioxidant enzyme activities such as CAT, total SOD, Cu/Zn-SOD, Mn-SOD and Fe-SOD and GPx was found in malathion-treated prepubertal male mice. Our findings have fully corroborated other work that proves that the administration of organophosphorus compounds to male mice caused an imbalance in antioxidant status in liver and kidney tissues [13]. Being the main actors of xenobiotic biotransformation, regulation of hepatic gene expression may play a central role in the adaptive response to altered metabolism by changing the capacity of enzymes in relevant metabolic pathways [21]. Thus, liver is the principal metabolizing site for mediating biotransformation of thiono-organophosphates and with kidney contributing to the elimination of toxic products [22,23]. Excessive generation of reactive oxygen species (ROS) and oxidative stress are the precursors of many pathologies associated to this organophosphorus exposure [12,25] many findings have reported the enhancement of oxidative stress in human OP poisoning cases [47] and in animals [12,48]. ROS such as superoxide anion, peroxyl radicals, hydroperoxyl radical, hydrogen peroxide produced from the molecular oxygen as a consequence of normal cellular metabolism [26]. At low or moderate concentrations, ROS are considered as part of normal oxidative metabolism, but at elevated concentrations, they cause tissue injuries, including lipids, proteins oxidation, DNA damage [27] and enzyme inactivation. They are also implicated in many pathological conditions such as cancer, diabetes, cardiovascular, pulmonary and autoimmune diseases, neurological disorder and aging, among others [48]. Recently, The clinical importance of this pathologies has led to the development of many pharmaceuticals and researches have already tested several natural compounds to prevent and protecting living organisms from the poisonous effects of pesticides [49,50,51]. On other hand, our findings, oxidative damages in both liver and kidney of treated mice was confirmed by a decrease of GPx-4 and GPx-3 mRNA expression respectively in liver and kidney. This decrease may be due to inhibition of enzyme activity after excessive free radical production. Moreover, free radicals attack not only proteins but also DNA bases; therefore, they have the potential to cause mutagenic lesions. These results were confirmed by RT-PCR analysis of enzyme mRNA levels. Gene expression of GPx-3 and GPx-4 were decreased in the treated group, indicating the disruption of the redox equilibrium in treated mice. The animals were administered malathion orally by gavage and the microscopic slide of selected organs showed major histological changes in their liver and kidney tissues according to previous study with diazinon [10]. Changes observed from light microscopes showed a varied scope of occurrence and different degree of intensity according to the dose of malathion. Results from histological investigation are in accord with diverse earlier studies which elucidate that the introduction to pesticides led to provoke intensive biochemical and physiological turbulence in experimental animals [10]. According to Tos-Luty et al. [52], malathion intoxication led to injurious effects on the organization of the liver and kidney with the persistence of thin subcapsular infiltrations, diffused parenchymatous degeneration of single hepatocytes. Furthermore, they indicated that the histopathological alteration in the kidneys occurred in all animals. These alterations demonstrated parenchymatous deterioration of the cells of renal tubules and hyperemia of the cortical area of the kidney, particularly of renal glomeruli, with infiltrations [10]. A number of experiments indicated that malathion caused testicular toxicity [16], hepatotoxicity [53], hematotoxicity [54,55], genotoxicity [56] and nephrotoxicity [54]. However, diverse works showed that malathion as well as other pesticides provoke histopathological alterations of liver and kidney in different rodent animals [57,58].

5. Conclusion

Taken together, it can be concluded that exposure to the organophosphorus malathion induced production of harmful ROS, causing thereby oxidative stress. Installation of oxidative stress can changed the body, liver and kidney weights, and altered their biochemical markers such as hepatic ALT, AST, PAL, LDH, total and direct bilirubin and renal plasma urea, creatinine and uric acid. In addition to the depletion of antioxidant enzymes system, malathion also induced molecular and histopathological modifications.

Conflict of interest

There is no conflict of interest.

Acknowledgement

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