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Hepatotoxicity and mitochondrial dysfunction after lead (Pb) exposure and the protective effect of fennel essential oil of this toxicity in Wister rats

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Abstract: *The present work focuses on evaluation of the toxic effects of an exposure to lead (Pb) on the liver mitochondria function in Wistar rats and the protective role of the fennel essential oils (FEO).*

The experiments were conducted on Wistar rats, which were treated daily through orally gavages during 21 days. 24 animals were divided into four groups of 6 rats each one: the control group received water, while group II received FEO (0.5 mg/kg); group III received Pb (25 mg/kg), and group IV received Pb + FEO in the doses of (0,5 + 25 mg/kg). The mitochondrial dysfunction induced by lead is expressed by reducing mitochondrial respiration (O₂ consumption) associated with an increase in mitochondrial swelling and permeability compared to the control group. A disturbance in the rates of oxidative stress biomarkers was also observed. This disturbance was expressed by an increase of the MDA rate and the Cytochromes C activity associated with depletion in the GSH rate and GPx activity compared to the control group. The rat's co-treatment with FEO restored all these parameters to values similar to the Control.

The whole of these results shows well that the exposure to the lead causes disturbances on the level mitochondrion, which are attenuated by fennel essential oils.

Keywords: Fennel essential oils, Lead, Toxicity, Mitochondria, Cytochrome

Résumé: Cette étude visait à évaluer les effets toxiques d'une exposition au plomb (Pb) sur l'activité du système antioxydant mitochondrial chez le rat Wistar et le rôle protecteur des huiles essentielles de fenouil (FEO).

Les expériences ont été menées sur des rats Wistar, qui ont été traités quotidiennement par gavage oral pendant 21 jours. 24 animales ont été divisés en quatre groupes de 6 rats: le groupe témoin a reçu de l'eau, tandis que le groupe II a reçu FEO (0,5 mg/kg); le groupe III a reçu du Pb (25 mg/kg) et le groupe IV a reçu du Pb + FEO aux doses de (0,5 + 25 mg/kg). Le dysfonctionnement mitochondrial induit par le plomb s'exprime par la réduction de la respiration mitochondriale (consommation d'O₂) associée à une augmentation du gonflement et de la perméabilité mitochondriale par rapport au groupe témoin. Une perturbation des taux de biomarqueurs du stress oxydatif a également été observée. Cette perturbation a été exprimée par une augmentation du taux de MDA et de l'activité des cytochromes C associée à la déplétion du taux de GSH et de l'activité GPx par rapport au groupe témoin. Le co-traitement des rats avec FEO a restauré tous ces paramètres à des valeurs similaires à celles du témoin.

L'ensemble de ces résultats montre bien que l'exposition au plomb provoque des perturbations au niveau mitochondrial, qui sont atténuées par les huiles essentielles de fenouil.

Mots clés: Huiles essentielles de fenouil, Plomb, Toxicité, Mitochondries, Cytochrome

Introduction

The Mitochondria are dynamic organelles that change their morphology in response to physiological signals and metabolic stresses [1]. It is play a significant role in the hepatocyte, as in other cellular types. Indeed, many ways and cellular processes are governed by mitochondria, like cellular death, the proliferation of the cells, and metabolism [2].

Lead is a naturally occurring bluish-gray metal found in small amounts in the Earth's crust and can be found in all parts of our environment. It is located in our food, water, air, and soil [3]. Humans can be exposed by the ingestion of lead-contaminated food or drinking water containing lead leaching from old corroded pipes, and dermal contact [4]. It is absorbed and conjugated in the liver then partially excreted in urine [5], while the rest usually accumulated in the brain, liver, spleen, kidneys, and lungs [6]. Lead usually induce adverse effects on the central nervous system as irritating, immunosuppressive, genotoxic, teratogenic, nephrotoxic, and other toxic effects on the hematopoietic system [7]. Lead is known to cause oxidative damage in various tissues by bringing about an imbalance in the generation and removing reactive oxygen species [8].

Fennel (*Foeniculum vulgare*) is an annual, biennial or perennial aromatic herb [9] flavouring and medicinal properties. Fennel has a variety of secondary metabolites which is used in the treatment of cancer, digestive irregularities, respiratory, and skin ulcers [10]. Besides, *Foeniculum vulgare* seeds have diuretic, antifungal, antioxidant, hepatoprotective, anti-inflammatory effects [11], Antidiabetic [12] and anti-cholinesterase activity [13].

This study aimed to evaluate the toxic effects of exposure to the lead on the activity of the mitochondrial antioxidative system in Wistar rats and the protective role of the fennel essential oils (FEO).

Material and methods

1. Chemicals

Lead acetate ($\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$) dissolved in water was distilled to make a solution of treatment at dose 25 mg/kg body weight/.

2. Plant material and extraction

The vegetable material used in this study consists of seeds of the fennel (*Foeniculum vulgare*). The fennel seeds were bought, in dried form, at an arborist. They are originating from the Wilaya of Tebessa (Algeria).

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus from crushed mature fruits. The content of essential oil in fruits of in *Foeniculum vulgare* was 0.26%.

3. Experimental design

The selected animal model is the albino rat Wistar, coming from the Pasteur Institute of Algiers (Algeria), weighing approximately 150 to 190 g at the beginning of treatment. The animals are divided into four groups of 6 rats each one.

- Group I (C): Control group received physiological water (1 ml).
- Group II (FEO): Received fennel essential oil (0.5 ml/kg).
- Group III (Pb): Received lead (25 mg/kg).
- Group IV (Pb+FEO): Received lead and fennel essential oil in the dose of (0,5 + 25 mg/kg).

4. Isolation of the liver mitochondrion

After the sacrifice, the liver mitochondrion was isolated, respecting the protocol described in Rustin et al. [14]. The livers were immediately taken and plunged in a plug of insulation TSE (250 mm sucrose, 50 mm sorting, 5 mm EGTA (Ethylene Glycol bis (-aminoethylether)-N, N, -Tetraacetic Acid), pH 7,2 to 4°C). The homogenates were centrifuged to 1300 rpm for 3 minutes at 4°C. The supernatants were centrifuged with 10,000 rpm at 4°C for 10 minutes. The final base made up of mitochondria [14].

5. Determination of swelling, permeability, and mitochondrial respiration

According to the method described by Rouabhi et al. [15], respiration was estimated using an Oxygraph (HANSATECH). We carried out estimating the mitochondrial permeability based on the rate of Ca^{++} traversing followed by an increase in mitochondrial size detected at 540 nm wavelengths for 3 min each 30s. According to the method of Kristal et al. [16].

6. Evaluation of Cytochrome C activity

Determination of cytochrome c level in liver tissue was performed using a commercial kit the cytochrome c assay kit (CYTOCOXI) uses an optimized colorimetric assay based on observation of the decrease in absorbance of ferrocytochrome c measured at 550 nm.

7. Evaluation of the statute oxidative mitochondrial

The procedure of Flohe (1984) measured the GPx activity [17]; the activity of GPX was measured at an absorbance of 420 nm.

Glutathione (GSH) concentration was measured according to Franco and Cidlowski [18]; 0.2 ml of a sulfosalicylic acid, centrifuged for 5 min at 1000 t/min, add 1 ml of Tris-HCl buffer + EDTA, pH 9.6, mix and add 0.025 ml DTNB dissolved in absolute methanol, and then measure the absorbance at 412.

The malondialdehyde (MDA) level was measured according to Warso and Lands [19]. The MDA was a product of lipid peroxidation and reacted with the acid's thiobarbituric reactive substance to produce a red-colored the complex with a maximum absorbance with 530 nm. Thus, 125 μl of supernatant were homogenized by sonication with 50 μl of PBS, 125 μl of TCA–BHT (trichloroacetic acid-butylhydroxytoluene) to precipitate proteins, and then centrifuged (1000 $\times$ g, 10 min, and 4°C). Afterward, 200 μl of supernatant was mixed with 40 μl of HCl (0.6 M) and 160 ml of TBA dissolved in Tris, and then the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was obtained at 530 nm.

8. Statistical analysis

The experiment data were analyzed using statistical software, MINITAB 17.1. Statistical analysis consists of a comparison of different treatments by ANOVA test.

Results

1. Study of mitochondrial apoptosis parameters

The results of Fig. 1 show a reduction in consumed oxygen concentration in the groups treated by Pb compared to control. Contrary, groups treated by the FEO alone or in association with the Pb show stability in the consumed oxygen concentration compared to control.

On another and, an increase in the mitochondrial permeability is recorded in the group treated with Pb, while these results do not show any significant variation in rats treated with FEO alone or in combination with Pb (Fig. 2).

Figure 1. Change in the lever mitochondria respiration (nmole) in treated groups and the Control

Figure 2. Change in mitochondria permeability after treatment

The results presented in Fig. 3, show a very highly significant increase in mitochondrial swelling after treatment with Pb [0.16 ± 0.002] compared to the Control [0.03 ± 0.037]. However, there were very highly significant changes in mitochondrial swelling in the group treated with combination of FEO and Pb [0.07 ± 0.009].

The effects of Pb on the activity of cytochrome-c are shown in Fig. 4. Cyt-c markedly increases in the group treated with Pb [0.08 ± 0.03] compared with the control group [0.01 ± 0.004]. There were no significant changes in Cyt-c in the groups treated with FEO alone or in combination with Pb [0.014 ± 0.002].

Figure 3. Changes in mitochondrial swelling of control and treated rats, $p \leq 0.000$

Figure 4. Effect of oral treatment of Pb and co-treatment of FEO for 21 days on Cyt-C

2. Effect on oxidative stress/antioxidant status of mitochondria

The statute oxidative mitochondrial was evaluated on the mitochondria level isolated from the liver from rats treated by Pb (25 mg/kg) and other by combination lead and fennel essential oils (0.5 ml/kg).

The rats treated by the Pb have presented a different significant reduction in the rate of mitochondrial GSH (1.85 ± 0.48 compared to the control (2.02 ± 0.36 pmol/mg)). There were no significant changes between Control and FEO + Pb groups (3.22 ± 0.57 pmol/mg) (Fig. 5).

The rats treated by the Pb presented a very highly significant reduction in the mitochondrial GPx activity (0.02 ± 0.039 pmol/mg) compared to the control (0.04 ± 0.01 pmol/mg). However, there were very highly significant changes in GPx in the group treated with a combination of FEO and Pb ($6.25 \times 10^{-5} \pm 0.014$ pmol/mg) (Fig. 6).

Treatment with Pb shows a no significant increase in mitochondria MDA [5.22 ± 1.76] activity compared to control (0.014 ± 0.01 pmol/mg). There were no significant changes between Control and groups treated by the FEO alone or in association with the Pb [1.97 ± 1.76] (Fig. 7).

Figure 5. Effect of oral treatment of Pb and co-treatment FEO for 21 days on GSH (nmol/mg) ($p \leq 0.05$) co-treatment FEO for 21

Figure 6. Effect of oral treatment of Pb and with days on GPx (Pmol/mg) ($p \leq 0.000$)

Figure 7. Effect of oral treatment of Pb and co-treatment with FEO for 21 days on MDA (nmol/mg)

Discussion

Lead is one of the most poisonous chemicals in the environment that can induce acute or chronic diseases [20]. It is known to cause oxidative damage in various tissues by bringing about an imbalance in the generation and removing reactive oxygen species [21].

In the present study, the lead's treatment caused increased mitochondrial swelling and permeability and decreased mitochondrial respiration. Indeed the leader shows an increase in Cyt. C. mitochondrial enzyme activities and tissue respiration may be altered due to the accumulation of lead in mitochondria [22]. The Pb toxicity is associated with an increased generation of the, which amplifies the cellular lesions via the oxidation and the deterioration of proteins. This study implied in the opening of PTPm, causing the massive swelling of the mitochondria and the collapse of the potential of the membrane and the synthesis of ATP, allowing the release of agents apoptotic (Cytc) [23]. These transitions from mitochondrial permeability also represent a brutal increase in the mitochondrial permeability [21].

Co-exposed FEO with Pb ameliorated these changes in mitochondrial swelling, respiration, and permeability. FEO's hepatoprotective effect might be due to the inhibition of oxidative stress-induced by Pb treatment [24].

According to the controls, the rats exposed to a subchronic dose of Pb decreased significantly the amount of GSH and increased the MDA level. These results are in concord with many results [25]. These two parameters are considered as the primary biomarkers of oxidative stress. Lead exposure can generate free radicals, resulting in the elevation of lipid peroxidation (MDA concentration) and reduced GSH level of hepatic, kidney, brain tissue [21, 26-28].

On the other hand, decreased activity of glutathione peroxidase (GPx) an in lead-treated rat. Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals, and lipid peroxides [21].

GPx is necessary antioxidant enzymes; it is a selenoenzyme that plays a significant role in reducing H_2O_2 and hydroperoxide to produce non-toxic products [27].

Our results reveal that FEO managed orally with the rats Co-treaty with the Pb protects the mitochondria by preserving the rate from GPx, GSH, and mitochondrial MDA. Antioxidants generally decrease the level of oxidation by transferring the hydrogen atom to the free radical structure [21].

The fennel HE can be associated with the effect antioxidant [9, 29, 30] of made up the bioactive contained in our extract, which is characterized by their capacities to restore the dysfunction mitochondrial by ensuring protection of the statute redox [31, 32] intra-mitochondrial. They can reduce the stress oxidative mitochondrial via the ERO's direct or indirect detoxification [32, 33].

FOE has a kidney protective effect against the damage induced by lead manager with the rats according to the results of El-Masry et al. [8]. El-Sheikh and Galal [30] also reported that pretreatment by the HE of fennel protects the organization from the rats against the hematotoxicity, the immunotoxicity, and the hepatotoxicity.

Conclusion

Through the previous results, we conclude that lead had a very bad effect on liver function in rats exposed to it at a dose of (25 mg/kg), and on the contrary. In addition, this study proved the fennel essential oils is very important in getting rid of the unwanted symptoms resulting from oxidative stress by the lead at a dose of (25 mg/kg).

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Compliances with ethical standards

Conflict of interest

Loubna Lemita, Belgacem Djabri, Salim Gasmi, Yousfi Amani, Rachid Rouabhi and Brahim Ben Aicha declare that they have no conflict of interest.

Ethical approval

The study was conducted following guidelines approved by the Animal Care and Use Research Ethics Committee (ISESNV) of the University of Larbi Tebessi (Approval number: ULT-ISESNV/2010/33). The conduct of the experiments, humane animal handling and welfare was guided by protocols contained in the publication of the National Institute of Health, “Guide for the Care and Use of Laboratory Animals.

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