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CURATIVE EFFECTS OF *MYRTUS COMMUNIS* LEAVES ON HEPATORENAL AND TESTICULAR TISSUES IN RATS INTOXICATED WITH CADMIUM CHLORIDE

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ABSTRACT

Myrtle (*Myrtus communis*, Myrtaceae) is a medicinal plant used in traditional medicine due to its therapeutic properties. The aim of this study is to test the prophylactic effect of both *Myrtus communis* hydromethanolic extract HME and Aqueous extract AE at a dose of 300mg/kg for 21 days.

The results of the present study indicate that chronic exposure of cadmium chloride for 60 days induce a decrease in body weights and organ weights (liver, kidney, testis and prostate) ($p \leq 0.05$).Thus, atomic absorption spectrophotometry (AAS) highlights a significant accumulation of Cd in the target tissues structures modifications compared with control rats. However, the administration of HME extract induce a considerable recovery of body weights, organ weights and positive change in target tissues compared with the aqueous extract AE. These results suggest that HEM extract can be used as a curative remedy to help patients suffering from cadmium exposure.

Key-words: Cadmium, Aqueous extract, Hydro-methanolic extract, *Myrtus communis*, SAA.

INTRODUCTION

Cadmium (Cd) is one of the rarest and non essential elements in nature. It is considered an environmental (air, water and soil) and industrial pollutant (Anses. 2012). Also, humans can be affected by this metal through its consumption in food such as cabbage, offal, rice, mother fruits and drinking water (Thompson, L. A., & Darwish, W. S., 2019). The oral absorption of this metal is about 5% and this rate of absorption can be increased during dietary deficiency of calcium, iron, zinc, copper and protein (Jarup, L., 2002). After absorption, it is transported with methalothionine which is a protein transporter and accumulated mainly in the liver and kidney. Cadmium is a highly toxic heavy metal (Bonet, A., 2011). It has a multiple and complex adverse effect overall human animal bodies (Nzengue, Y., 2008). This metal can cause local problems such as bone deformity, organ atrophy, hepatic-renal and testicular necrosis. At the systematic level, through the cumulative effect of Cd, it can cause disruption which is associated with cellular alteration such as denaturing of proteins, cleavage of DNA molecule, etc. Long-term Cd environmental exposure can lead to carcinogenic reactions in humans (Achanzar, W.E and *al*., 2001; Luevano, J., & Damodaran, C., 2014).

Today there is a lot of interest in herbal treatments against heavy metal poisoning. The leaves of the *Myrtus communis* plant have an important source of therapeutic molecules due to their bioactive compounds. The plant *Myrtus communis* L., from the Myrtaceae family is known for its antiseptic, disinfectant and astringent properties (diarrhea, dysentery) as well as for its hypoglycemic effect, and the treatment of the urinary and respiratory tracts diseases (Baba Aissa, F., 1999; Mimica-Dukić, N and *al* .,2010).

The present study aim to find and select one of the two extracts (HEM, AE) of the MC leaves that possesses better curative effect on the physiological and histological aspects of cadmium intoxicated rats organs.

MATERIEL AND METHODS

Plant material

Myrtus communis plants were collected in January 2021, in the Mediterranean region located on southeast of Blida, Algeria. This plant species has been authenticated according to Professor Mohamed Terras. The collected leaves are dried and crushed with an electric grinder until the obtaining of fine powder which has been conserved in the dark at 4°C.

Extracts preparation

A. Aqueous extract (AE)

The aqueous extract of the plant was obtained by the infusion of 100g of leaf powder in 2 litters of distilled water, heated to 100°C under stirring for 24 hours. The mixture was filtered through a Watman paper, and then the filtrate was evaporated at 45°C using rotavapor until complete evaporation. The obtained dry extract was stored at 4°C until use (Millogo-Koné, H and *al*.,2012).

B. Hydromethanolic extract (HME)

The hydromethanolic extract of *Myrtus communis* leaves was prepared from 100g of leaf powder which was macerated in 1 litter of hydromethanol (20/80), at room temperature in the dark for 24 hours. Then, the solution was filtered through Watman paper and the obtained solvent was separated from the filtrate using a rotavapor and then in the oven until complete evaporation of the solvent at 45°C. The resulting extract was conserved at 4°C until use (Spigno, G., & De Faveri, D.M., 2007).

Animals and treatment

Male *Wistar* rats obtained from the Department of Biology, Faculty of Science and Technology, Dr. Moulay Taher University of Saida have been used on the present experimentation. The animals received food and water *ad libitum* in an animal house (12:12 h light/dark cycle) for one week in order to be acclimatized. The 28 rats were randomly divided into four groups, containing seven animals each.

Groups' repartition

Group 1 (control): received 1 ml of distilled water by gastric gavage.
Group 2 (Cl2Cd): received 1.8 mg/ml of cadmium chloride for 60 days.
Group 3 (Cd_HME): received 1.8 mg/ml of Cd for 60 days and then 21 days by HME extract at the dose of 30mg/ml [12].
Group 4 (Cd_AE): received 1.8 mg/ml of Cd for 60 days then 21 days of AE extract at the dose of 30mg/ml.

Determination of cadmium levels in tissues

The determination of Cd in the target organs (Liver, Kidney, Testis and Prostate) was performed by electro thermal atomic absorption spectrophotometer (EATAS), equipped with a Zeeman correction type Analyst 800 Perkin Elmer with atomization furnace type THGA and cadmium lamp type EDL. The mineralization of the organs is performed by introducing 50 mg of the sample into the Teflon digestion bomb. Then, 0.5 ml of the mixture of nitric acid (NHO_3) and hydrochloric acid (HCL) 5/1 (V/V) was added to the sample. Latter was put in the oven at 170 C° for 5 hours. Then, the residues of our sample were dissolved in the diluted solvent (NHO_3+ HCL+ HO_2). The measurement of cadmium content was performed on the wavelength at 228.8 nm, the power supply of the cadmium lamp is 230 mA and the used carrier gas is Argon.

Histological study

This study was carried out in the laboratory of pathological anatomy of the hospital of Tiaret. After fixing organs: the liver, kidneys and testes in formalin (10%) for 7 days. Using a model dehydration machine (Leica TP1020), the samples were passed through three successive ethanol baths. They were then diluted in two xylene baths and impregnated in two paraffin baths. After that, samples have been embedded in paraffin wax so that the cut can be made. The resulting block is then cooled. Tissue blocks were prepared for sectioning at 4 μm. Then the tissue sections were deparaffinised and stained with hematoxylin and eosin (Bancroft, J. D., & Gamble, M., 2008).

Statistical analyzes

Data were expressed as means with standard error (M ± ESM). The comparison between the treatment and control groups was made by ANOVA followed by Tukey multiple comparison and using Student's t test. The level of significance was set at *p 0.05*.

RESULTS

Table 1 shows the variation in body weight in control and experimental rats. Daily administration of cadmium salt (Cl_2Cd) at a dose of 18mg/kg for 60 days causes a significant decrease in the body weight of rats (G2) compared to controls group, and a significant decrease in target organs wheighs namely Liver and Testes. On the other hand, the ingestion of *Myrtus communis* leaf extracts (HME and AE) on fourth and fifth groups respectively, shows a positive improvement in the physiological status of the experimental animals (body weight and organ weight) compared to the Cd poisoned group.

Table 1: Body weights (g) and organ weights (g) of the groups (control, Cd-exposed rats and rats treated with EHM and EA extracts after Cd intoxication).

		Group 1 (control)	Group 2 (Cl2Cd)	Group 3 (Cl2Cd-EHM)	Group 4 (Cl2Cd-EA)
Body weight (g)		219.05± 0.19	173.22 ±1.96*	196.72 ±2.43	207.65± 1.86
Organ weight (g)	Liver	9.91 ± 1.44	5.03 ± 0.87*	11.10 ± 0.43	10.81 ±0.88
	Kidneys	2.20 ±0.18	1,52 ± 0.14	1.93 ±0.33	2.54 ± 0.40
	Testis	3.07 ± 0.06	1.43 ± 0.31*	2.35 ± 0.15	3.51 ± 0.13
	prostate	1.74 ±0.39	0.35 ± 0.18	1.33 ±0.17	1.82 ±0.49

Table 2 shows that Electrothermal atomic absorption spectrophotometry revealed high concentrations of cadmium in the Cd intoxicated rats compared to the control group. However, the 21 days treatment with MC leaf extract (HME and AE) in 3 rd and 4th groups

respectively, has been able to reduce the concentration of cadmium in target organs (liver, kidney, testes and prostate).

Table 2: Evaluation of cadmium levels in tissues by SAAE.

	Group 1 (control)	Group 2 (Cl2Cd)	Group 3 (Cl2Cd-EHM)	Group 4 (Cl2Cd-EA)
Liver µg.g-1	17.28	45.03	105	12.70
Kidneys µg.g-1	11.20	38.6	8.9	15.4
Testis µg.g-1	10.27	29.8	7.6	13.8
prostate µg.g-1	15.10	25.5	6.6	13.2

The histological study of the target tissues (kidneys, liver and testes) showed a normal tissue architecture in the control group (G1) (fig. 1, fig. 2, fig. 3). On the other hand, the administration of cadmium chloride in the G2 group showed numerous alterations namely a renal lesion (fig. 1 G2) represented by vascular congestions and glomerulus dissimulation. Similarly, the hepatic inflammatory response was observed in Cd intoxicated group (fig. 2 G2), on which we highlighted the presence of tissue destruction, leukocyte infiltration and hyperchromatic nuclei in the hepatocytes. Thus, testicular tissue (fig. 3 G2) showed a disorganization of seminiferous tubules, disturbance on spermatogenesis cells, absence of spermatozoa and destruction of interstitial Leydig tissue. However, histological sections of the kidney, liver and testes of rats treated with *Myrtus communis* leaf extract (HME) for 21 days objectivise structures comparable to those of normal rats. The glomeruli are well defined, the liver tissue is normal and the seminiferous epithelium is well developed with a sperm-containing lumen. In contrast, tissue sections from rats treated with aqueous extract (AE) of MC leaves for 21 days show accumulation of unconjugated bilirubin in hepatocytes and testicular necrosis.

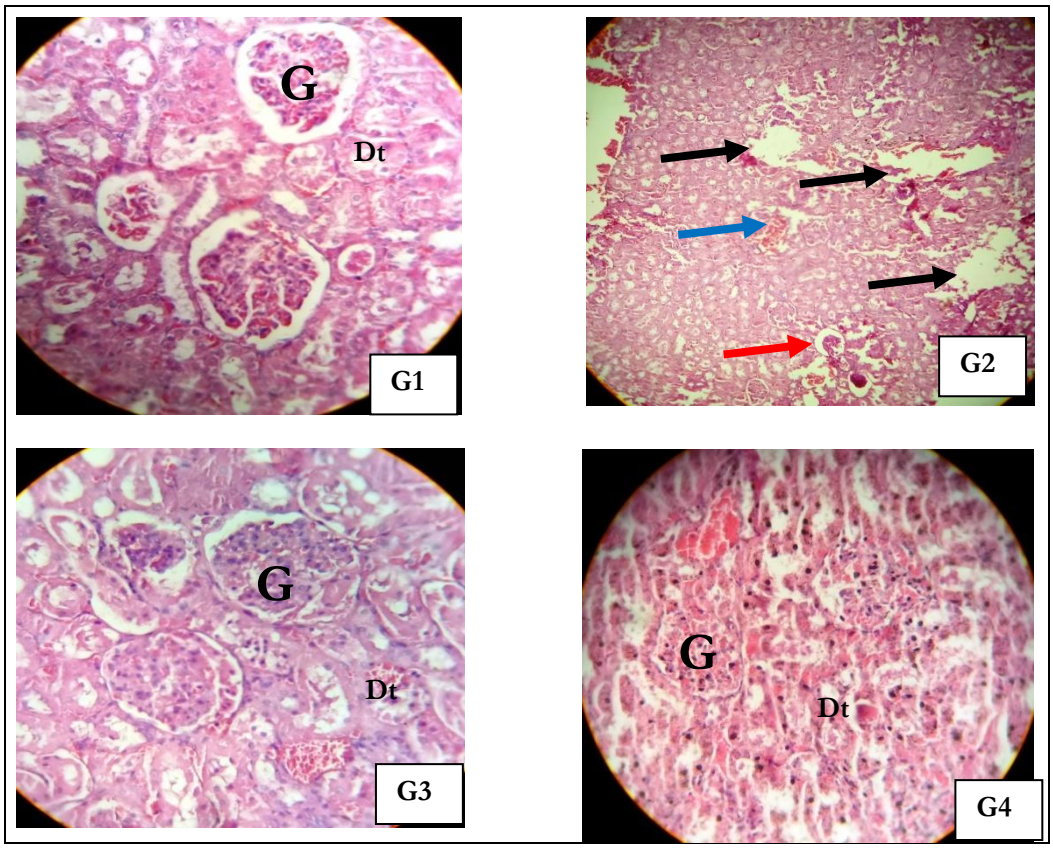

Figure 1: Histological sections of rat kidny with hematoxylin and eosin (Gx 40; except G2 with Gx 10); (G1) normal architecture and normal glomeruli in the control group. (G2) (Cl2Cd), (Intoxicated animals) showing the vascular congestion (blue arrow), dissimulation of the glomeruli (red arrow) and tissue destruction (black arrow). (G3) and (G4) treated with HME and AE extract respectively , Showing the regeneration of the majority of the glomeruli and distal tubule like the control.

G: Glomeruli; Dt: Distal tubule

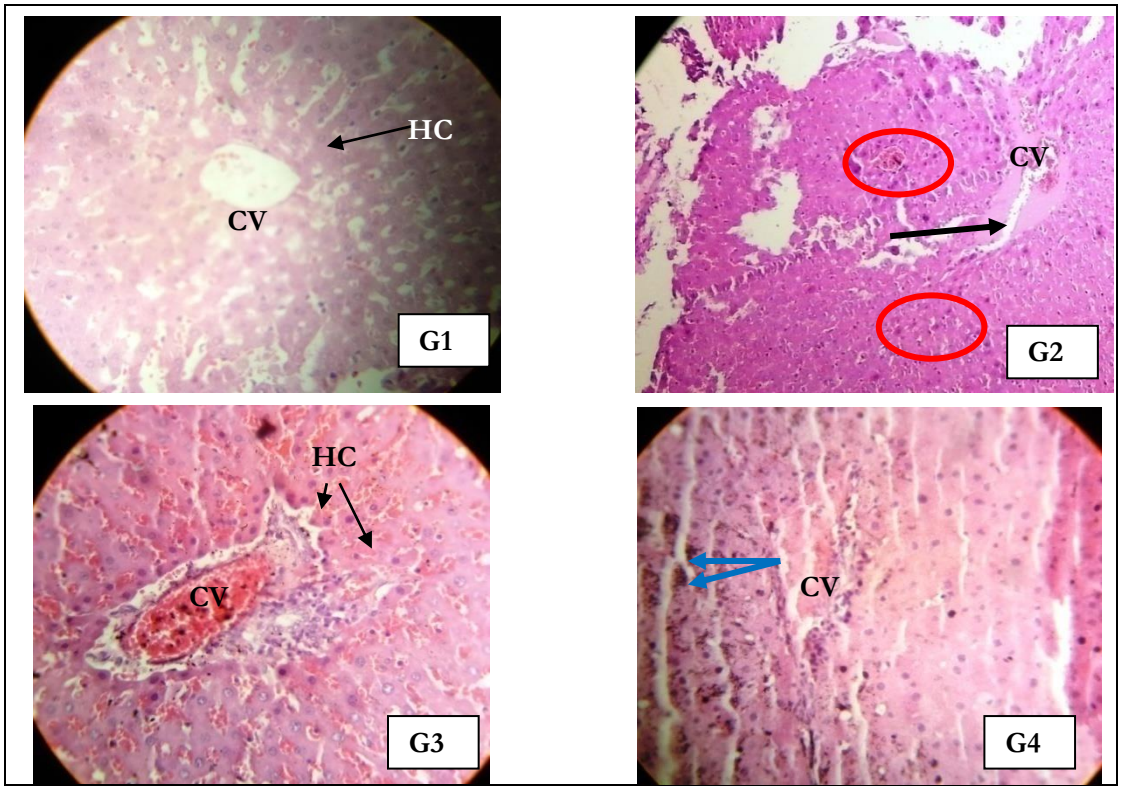

Figure 2 : Histological sections of rat liver with with hematoxylin and eosin (Gx 40; except G2 with Gx 10, to observe the general aspect of the liver tissue), (G1) liver of control rat showing the normal histological appearance including (CV), hepatic cells (HC). (G2) (Cl2Cd)(Intoxicated animals) showing the nuclei of atypical hepatocytes (red circle), vein thrombosis (black arrow) with inflammation, vascular congestion and tissue destruction. (G3) showing the normal tissue section like that of the control. However, (G4) showing normal tissue marked by intrahepatocyte of accumulation bilirubin (blue arrows).

CV Central vein; HC: Hepatic cells.

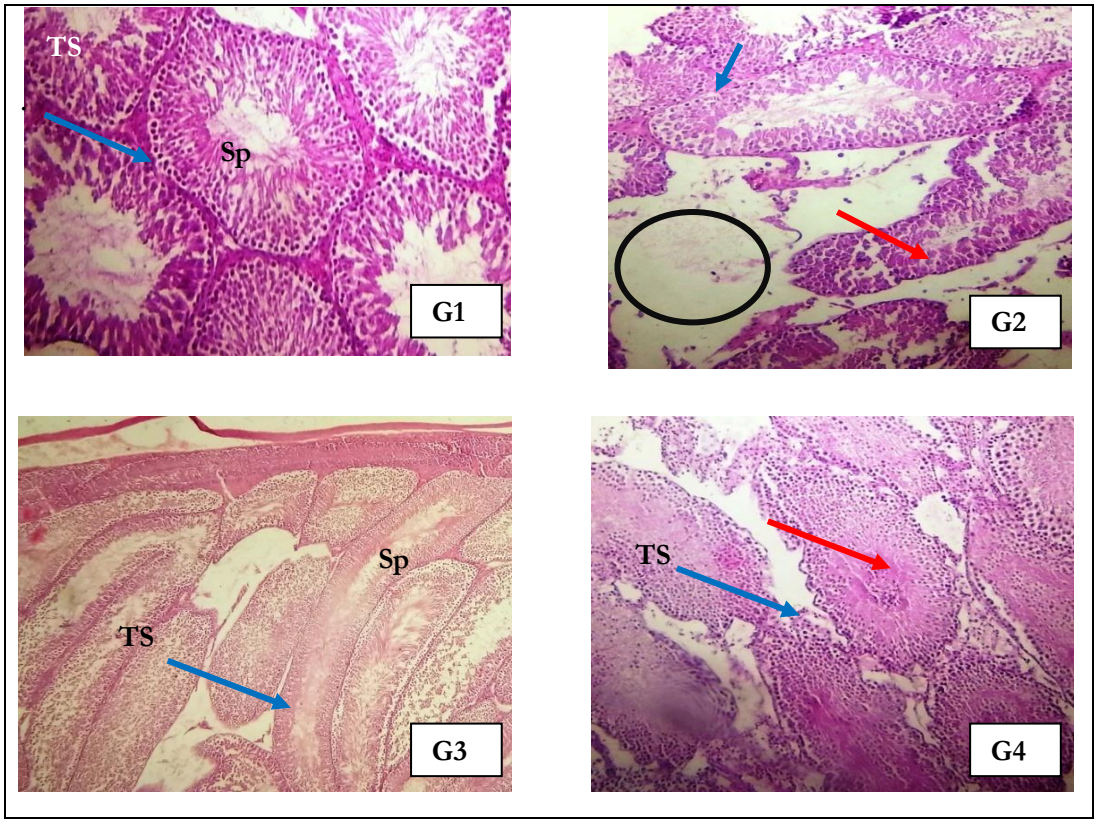

Figure 3: Histological sections of rat testis with hematoxylin and eosin (Gx40). (G1) normal rats with normal seminiferous tubules. (G2) Rats received Cl2Cd only show increased spermatozoa numbers (red arrow) and showing the majority of the seminiferous tubules are disorganized, destruction of the interstitial tissue of Leydig (black circle) with vascular congestion. (G3) EHM-treated rats showing almost normal seminiferous cells. However, (G4) showing subnormal tissue marked by irregularity of the tubules (TS), show increased spermatozoa withe vascular congestions in the tubule center and large interstitial spaces.

TS: seminifer tubule; Sp: spermatozoa.

DISCUSSION

The treatment against harmful and deleterious effects of heavy metals and particularly cadmium is considered as a non resolved heath problem. Unfortunately, this is a problem that has not been completely and acceptably resolved. This study was carried out to evaluate and compare the impact of the two types of *Myrtus communis* leaf extracts after chronic cadmium chloride poisoning.

The obtained results prove that cadmium affects the increase of body weight and causes atrophy of organs (liver, kidneys and testicles). Our results are consistent with the findings of Diaby and *al*.,2016. Otherwise, the decrease in organ weight could be explained by cellular atrophy and/or reduced cell multiplication due to cadmium affecting cell activity, proliferation, differentiation and consequently leading to their apoptosis and organ atrophy could be related to the slowing down of body weight observed by some researchers (Onwuka, F.C and *al.*,2010; Al-Gehani, S.A. 2013).

In addition, SAAE analysis has revealed significant accumulation of cadmium in target organs. This assessment is similar to the work of Demenesku and *al*.,2014. Several studies show that cadmium is highly concentrated in the liver and kidney (between 50% and 70% of the total load) where the levels of transporter proteins metallothioneins (MT) are higher (Il'yasova, D., & Schwartz, G.G., 2005). In this case, MT play a role in the initial distribution of Cd to different tissues such as glands and accumulation according to their metallothioneins levels. In the same competition, chronic exposure to cadmium for 60 days, causes tissue changes in the studied organs (glomerular damage, inflammation and destruction of liver tissue and disorganization of seminiferous tubules with depreciation of spermatogenesis), our results are similar to the works by several authors (Albasha, M.O., & Azab, S.A., 2014; Mouro, V.G and *al*.,2019; Khudhair, A.D., & Abass, D.A., 2019). These alterations are probably related to cadmium toxicity that induces the tissue damage and metabolic disturbances.

Therefore, administration of *Myrtus communis* (MC) leaf extracts, either the hydromethanolic extract (HME) or the aqueous extract (AE), after cadmium intoxication in rats, recovered their body and organ weights, which could highlight the beneficial effect of

the plant. This is probably due to the effect of phenolic components of myrtle leaves that stimulate the correction of metabolic mechanisms. In addition, SAAE analysis revealed that the hydromethanolic extract (HME) of *Myrtus communis* leaves reduced the level of cadmium in the target organs better than the aqueous extract (AE). Unfortunately, there is no study elucidating the mechanisms of heavy metal detoxification by *Myrtus communis* leaf extract. These results could be explained by the stimulation of transporter proteins (metallotheionin) to release presumed bound cadmium or by the activation of antioxidant enzymes. Indeed, a previous study assumed that *Myrtus communis* leaves possess two properties: modulation of certain enzymes (hexokinase, aldose reductase, phospholipase C, protein kinase C, cytooxygenase, lipoxygenase, myeloperoxidase, NADPH oxidase, and xanthine oxidase) and antioxidant activity (EL-Bahay, C., 1999).

On the contrary, administration of the hydromethanolic extract (HME) corrected the appearance of the tissues (liver, kidney and testis) as compared to the controls. In addition, treatment with aqueous extract (AE) of *Myrtus communis* leaves revealed an accumulation of non-conjugated bilirubin (pre-hepatic jaundice) and irregularity of seminiferous tubules. These results could be related to the ratio (dose/effect) of each extract. Indeed Hayes, A. W., & Loomis, T. A. (1996) founds according to the LD50 that the aqueous extract was more toxic than the alcoholic extract. These observations suggest that this dose of 300mg/kg/d of the aqueous extract (AE) of *Myrtus communis* leaves would have a toxic effect. Hosseinzadeh and *al*, (2011). revealed the LD50 of aqueous and ethanolic extract of *Myrtus communis* 473mg/kg and 790 mg/kg respectively. Concerning the histological study of the prostate, we found an alteration of this biological tissue in all the groups that does not allow to distinguish four lobes (dorsal, lateral, ventral and anterior) and this could be due to one of the artifacts of the histological technique and we left this part for next studies.

3 Conclusions

On the light of these results, chronic exposure to cadmium affects the metabolic and reproductive functions of male rats. The hydromethanolic extract of *Myrtus communis* leaves is able to recover the physiological status of rats, decreasing the accumulation of cadmium in tissues and correcting the target tissues in comparison with the aqueous leaf extract (AE). The valorization of the hydromethanolic extract of the leaves of *Myrtus communis* could be considered as an alternative solution to the synthetic drug in people with risk of contamination by heavy metals like cadmium. Through histological observation under the microscope, it is proven that 300 mg/kg of the aqueous extract can cause adverse effects.

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Author Contribution: this manuscript contains complementary results of my doctoral research work. I worked on data collection, interpretation, manuscript final writing, editing and approval. BA my thesis supervisor contributed to the correction the article. ZM performed the processing of my article. AT verified the analytical methods and BB carried out the histological study

Conflicts of Interest: The authors declare that they have no conflict of interests regarding the publication of this article.

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