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The therapeutic effect of *Cinnamomum cassia* essential oil against hepatotoxicity induced by co-exposure to lead and manganese in developing Wistar rats

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ABSTRACT

The present study was designed to evaluate the protective effect of *Cinnamomum cassia* essential oil (CCEO) on hepatotoxicity induced by co-exposure to lead acetate (Pb) (2.84mg/ml) and manganese chloride (Mn) (4.79mg/ml) in developing Wistar rats. After weaning, rats exposed to Pb-Mn received injections of *Cinnamomum cassia* essential oil (0.1 ml/kg) for 21 days. Extraction by hydrodistillation yielded 1.10% of OECC and the characterization of this oil by GC-MS indicates that the major components of this oil are: E-cinnamaldehyde (85.77%), linalool (3.70%), Z-cinnamaldehyde (3.22%) and B-phellandréne (1.19%). The concentration of lead and manganese in hepatocyte tissues was significantly increased compared to control rats. However, levels of hepatic markers such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) were significantly increased in blood in Pb-Mn poisoned rats. Pb-Mn-induced oxidative stress in liver tissue was indicated by decreased levels of superoxide dismutase, catalase and glutathione peroxidase respectively. Histologically, the liver showed several tissue alterations such as hepatocyte necrosis and the presence of steatosis foci. Administration of OECC considerably attenuated previous biochemical alterations as well as histological and cellular changes in liver tissue. In this study, we can conclude that the *Cinnamomum cassia* essential oil showed a hepatoprotective effect.

Keywords: Essential oil, *Cinnamomum cassia*, CG/MS, Hepatotoxicity, Lead acetate, Manganese chloride.

INTRODUCTION

Lead is an environmental and industrial pollutant. Its presence in human and animal tissues has often been associated with considerable health risks[1]. It alters the biology of the cell by disrupting many metabolic pathways and different physiological processes. It can alter certain neurobiological mechanisms that play a critical role in brain development[2].

The manganese is at the same time an essential element for the living being and a toxic metal, considered as one of the most abundant elements on the terrestrial crust, indeed it is found in rocks, soil, water and food[3]. It has been discovered for centuries and used for various industrial applications. This element is recognized for its primary role in many enzymatic reactions as a cofactor or activator of numerous enzymes mainly localised in the brain as well as a regulator of the metabolism of lipids, proteins and amino acids in the organism[4].

Although, for many toxic metals, there are effective detoxification chelators available. In parallel to the use of modern and especially synthetic drugs, it is also possible to use alternative and traditional medicine.

However, products like secondary metabolites by plants, essential oils intervene in many domains, they have found their place in aromatherapy, pharmacy, cosmetics and food preservation[5]. For decennia, *Cinnamomum cassia* has been used for its culinary and medicinal virtues. Other properties have been discovered, such as its anti-inflammatory, antioxidant, antimicrobial and anti-carcinogenic effect[6].

In the light of these data, our study aims at investigating the effect of the *Cinnamomum cassia* essential oil of bark intoxicated by lead acetate and manganese chloride on the liver in wistar rats during the development period (gestation and lactation).

MATERIALS AND METHODS

Extraction and determination of the chemical composition of the essential oil by GC/MS :

The cinnamon bark "*Cinnamomum cassia*" was bought and imported from Indonesia, then identified by taxonomic experts (Pr sitayeb). The sample was conserved, and the specimen voucher, coded P-200986, was deposited in the herbarium of the Biology Department of the Faculty of Science of the University of Saida, Algeria, for future reference. The analytical study of the essential oil of "*Cinnamomum cassia*" was carried out by gas chromatography type VARIAN CHROMPACK - CP 3900 by injection of 0.1 μl of extract. The carrier gas used is helium He at a flow rate of 1.2 ml/min. The column used is a CP type capillary column. Chrivasil-Dex CB Fusedsilica, 30 m long and 0,25 mm inner diameter. The thickness of the stationary is 0,25 μm; the temperature of the initial injection column is programmed at 70 ºC for 2,50 min, then rises in steps of 5 ºC/min at 280 ºC; the detector used for this analysis is a mass spectrometry type detector (Saturn 2200) with a temperature of 280 ºC. The instrument is controlled by a menu-driven computer with software suitable for this type of analysis and a NIST database for compound identification.

Preparation of the CCEO injection solution:

A dose of 0.1 ml/kg of essential oil (EO) of *Cinnamomum cassia* [7] was diluted in sterile bidistilled water (100 μL) with one drop of tween 80 [8].

Preparation of the oral solution:

Lead acetate and manganese chloride tetrahydrate ($MnCl_2$ $4H_2O$) are solubilised in bidistilled water at 2.84 and 4.79 mg/ml respectively[9].

Distribution of Lots

At day 0 of mating the rats are separated into two groups:

Group Mn-Pb: represents the rats co-exposed to manganese ($MnCl_2$) and lead acetate ($C_4H_6Pb2H_2O$) which receive them in bi-distilled water orally from the first day of mating until weaning.

Group C (control): consisting of rats which receive bi-distilled water

At 21 days after giving birth, the rats are separated into 3 groups:

Group C (control): made up of young rats (n=7) from the same mating which receive drinking water.

Group Pb-Mn: consisting of young rats (n=7) from the first mating which are given manganese chloride and lead acetate in bi-distilled water with physiological water administered intraperitoneally for 15 days after weaning.

Group Pb-Mn treated with CCEO: consisting of young rats (n=7) from the same mating which receive Pb-Mn in bidistilled water with the injection solution administered intraperitoneally for 21 days after weaning.

Body and liver weight

The body weight of each animal was daily recorded throughout the duration of the experiment. The liver weights of different groups of animals were registered.

Biochemical tests

The lead and manganese analysis in tissues of liver

Depositing 1g fresh weight of each sample with 1 ml of nitric acid (HNO_3) at 65% purity, we bring the temperature to 95 ° C for one hour, after cooling; we supplement the content to 4ml of double distilled water. The lead and manganese concentrations were determined in the liver by atomic absorption spectrophotometry (SHIMA DZU AA6200) and the values were expressed in μg /g.

Determination of liver parameters

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) according to the methods of Reitman and Frankle[10]. Determination of the concentrations of albumin, cholesterol and triglycerides by following the methods of Thomas[11] and Fossati et Prencipe[12] respectively.

Measurement of antioxidant enzyme activity

The rat liver was weighed and homogenized in a buffer solution containing 0.32 M sucrose, 0.5 mM EDTA, 10mM Tris-HCl (pH 7.4) in ice (1mg tissue /4 ml buffer solution) using a glass/glass homogenizer. The fabrics were maintained at 4°C during all dissection and homogenization procedures. The homogenate was centrifuged at 1000xg for 15 minutes at 4°C. The supernatant thus obtained was centrifuged at 10000xg for 15 minutes at 4°C. The pellet constitutes the mitochondrial fraction and supernatant is re-centrifuged at 10,000g/30 minutes Both pellets thus obtained are solubilized in a buffer solution containing 0.32 M sucrose, 0.5 mM EDTA, 10mM Tris-HCl and 0.02% digitonin (pH 7.4), digitonin is added to release all imprisoned mitochondria in the synaptosomes and centrifuged a second time at 10000×g for 15mn at 4°C, the pellet thus obtained constitutes the fraction of the total mitochondria which will be solubilized in a solution containing sucrose (0.32 M at pH 7.4)[13]. Super oxidize dismutase (SOD) (EC 1.15.1.1) was analyzed on supernatant using the technique of Wenqiang et al .[14]; this method is based on inhibition of formation of adenine nicotinamide dinucleotide, phenazine methosulfate and formazan amino tetra zoliumblue. Activities and levels of antioxidants at the brain level such as catalase (CAT), glutathione peroxidase (GPx) were analyzed by the Karousou et al .[15]; Jollow et al.[13] methods respectively.

Histological study

Samples of liver spleen were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Paraffin sections (5μm thick) were prepared, routinely stained with hematoxylin and eosin (H&E) dyes, and then examined microscopically[16]

Expression and statistical analysis of results

The results are expressed as the mean (M) of the individual values, assigned from the standard error to the mean (SEM). The comparison of several means is carried out by an analysis of variance (one wayAnova) with the intoxication factor (Pb-Mn, C). Repeated-measure Anova were used for time factor analysis. A probability p < 0.05 is considered significative. Statistical analyses were performed using Sigma Stat software (SPSS Inc., Chicago, IL, USA).

RESULTS AND INTERPRETATIONS

The yield of essential oil

After hydro-distillation of the *Cinnamomum cassia* plant material. The yield obtained by the report between the weight of oil extracted and the weight of plant matter used, expressed as a percentage (%) is equivalent to 1.1%.

Principal Essential Oil Compounds Detected GC/MS

The analysis of the essential oil of *Cinnamomum cassia* by gas chromatography identified 14 major compounds listed in Table 01 in order of elution.14 components representing the sum of the percentages of the components obtained were identified of which 88.995 % are aromatic aldehydes, 6.148 % are monoterpenic hydrocarbons, 4.077 % are terpenic alcohols and 0.780 % are phenols. The major components of this oil are: E-cinnamaldehyde (85.77%), linalool (3.707%), Z-cinnamaldehyde (3.22%) and B-phenol (1.19%).

Effect of Pb-Mn on body and liver weight:

The body weight results show that animals co-exposed to Pb-Mn showed a significant ($p < 0.05$) decrease in body weight compared to control animals during the experiment. Animals exposed to pb-Mn and treated with OECC showed a significant ($p < 0.05$) increase in body weight compared to untreated poisoned rats (Table 02). The results found in the poisoned animals also showed a significant ($p < 0.05$) decrease in liver weight relative to controls (Table 02). In contrast, animals that were treated with OECCs showed a significant ($p < 0.05$) increase in liver weight compared to poisoned rats.

Biochemical determination

Determination of lead and manganese levels in liver tissue

The results obtained from lead and hepatic manganese determinations using atomic absorption spectrophotometry (AAS), are significantly ($p < 0.05$) increased in Pb-Mn poisoned rats. These levels are decreased following administration of the plant extract in the batch of previously intoxicated rats (Pb-Mn-CCEO) (Table 03).

Liver function

Biochemical analysis of liver biomarkers at the end of the experiment showed significant elevations in ALT, AST, total bilurbine and alkaline phosphatase in rats exposed to Pb-Mn compared with controls (Table 03). After the duration of treatment with OECC, there was a marked correction in plasma levels of these liver parameters in previously intoxicated rats.

Liver activity of oxidative status enzymes

The liver oxidative status results show a significant decrease in liver SOD, CAT and GPx in rats exposed to Pb-Mn during the developmental period compared to control rats. While a significant elevation is observed ($p \leq 0.05$) under the action of OECC in the treated lot following intoxication (Table 04).

Effect of Pb-Mn on the structural architecture of the liver

Histological sections of the livers of Pb-Mn intoxicated rats show necrosis and hepatic parenchyma with swelling, ballooning of hepatocytes and lipid vacuoles in the hepatocytes producing foci of steatosis (Figure 01.B) compared to the livers of control rats.

Similarly, the histopathological study of liver samples from intoxicated rats showed a striking feature as shown in Figure 01 (B1):

- Inflammatory infiltrate around the portal spaces.

The treatment of rats exposed to Pb-Mn with C. cassia EO revealed a hepatic parenchyma with portal inflammation. In addition, the hepatocytes showed signs of regeneration: large binucleated hepatocytes with granular cytoplasm (Figure 01.C).

DISCUSSION

Our results show that EO from *Cinnamomum cassia* obtained by hydrodistillation has a yield of 1%, which is in accordance with the work of [17]and [18] who reported a yield between (0.4-4.9%). In addition, the GPG-MS of this condiment contains primarily an essential oil with various chemical components of various natures such as E-cinnamaldehyde (85.775%), linalool (3.707%), Z-cinnamaldehyde (3.22%) and Eugenol (0.48%) these results corroborate with those of Sariözkan et al. 2016 who show that E-cinnamaldehyde has a percentage of 88.2% in their study.

Impact of lead and manganese on biochemical parameters

The use of atomic spectrophotometry allowed us to record high blood lead levels in rats co-exposed to Pb-Mn during gestation and lactation. These results show a good impregnation of lead, which means that the liver is one of the preferred sites for lead binding[19].

In addition, the level of Mn in tissues is an effective biomarker and representative of the exposure of the latter. The amount of Mn is highest (about 66%), with a half-life of 37 days[20]. The level of Mn found in this study is significantly elevated in poisoned rats compared to control rats. However, studies have shown that during pregnancy, blood levels of Mn increase over the three semesters and Mn enters the placenta via active transport[21] the increase in Mn levels during pregnancy may also be related to accelerated erythropoiesis, intestinal absorption, or tissue mobilization of Mn[22]. Tholin et al.[23] also reported that Mn levels during pregnancy increase with each trimester.

Effects of Pb-Mn on liver function tests

ALT and AST are sensitive indicators of acute hepatic necrosis due to Pb [24]

Through our study a significative increase in ALT and AST levels in Pb-Mn poisoned rats during pregnancy and lactation was observed compared to control rats, which could explain why Pb binds to plasma proteins, where it causes alterations in a large number of enzymes. It may also disrupt protein synthesis in hepatocytes [25].

However, the liver is an important target for lead. Lead has a high affinity for protein thiol groups in liver cell membranes, leading to liver necrosis and the output of ALT and AST in serum. These two parameters are indicative of cell leakage and loss of functional integrity of the hepatocyte cell membrane. This result is confirmed by the work of [26]. Our results are in agreement with the authors who reported that lead has a hepatotoxic effect [27].

In fact, Upadhyay et al.[28] demonstrated that rats nourished with lead contaminated food experience oxidative stress related to elevated serum ALT and AST and alkaline phosphatase (PAL) levels. This phenomenon is due to the accumulation of amino acids such as alanine and glutamic acid resulting from the degradation of somatic proteins. These amino acids are transformed under the action of aminotransferases into ketone bodies and then into glucose (neoglucogenesis).

In addition, the liver is a critical organ for the regulation of Mn homeostasis in the body. Furthermore, Mn toxicity is

rare and manifests itself mainly in the liver, probably due to the primary role that this organ plays in eliminating Mn from the blood through biliary excretion [29]. Consumption of Mn-contaminated water causes the liver to overflow, which can lead to liver damage[30].

The work of Fordahl et al.[31]. indicates that a wide exposure to Mn has an effect on hepatic metabolites, mainly by causing disturbances in lipid metabolism and thus the excessive formation of oleic acid, hydroxybutyric acid and ketones, the latter being in surplus in the blood will be eliminated in the urine, adding that alterations in hepatic metabolites were correlated with the tissue increase in manganese and the decrease in iron levels.

Exposure to Pb-Mn during gestation and lactation increases total bilirubin and alkaline phosphatase levels in poisoned animals compared to control animals. This is similar to the work of Nabil et al.[32]. Which justify this elevation by the induction of the enzyme heme oxygenase which converts heme into bilirubin, the latter being transported in the liver as a complex with the serum. Then, bilirubin is conjugated with glucoronide in the liver under the toxic effect of lead, this conjugation with bilirubin renders it inactive. In addition, bilirubin has a protective role against metal-induced oxidative damage to the cell membrane[33].

In addition, the evaluation of the toxicity of Pb and Mn was expressed by the assay of antioxidant enzymes, namely CAT, GPx, SOD. Knowing that these antioxidant enzymes help maintain the homeostasis of redox potential[34].

The analysis of tissue antioxidant status indicated that Pb-Mn decreases the enzymatic activities of catalase (CAT), superoxide dismutase (SOD) and Glutathione peroxidase (GPx), leading to a dysfunction of the antioxidant defense system. Therefore, Pb can affect the antioxidant barrier by inhibiting the functional thiol groups of enzymes such as SOD [35]. This metal has been reported to alter the antioxidant system of tissues in proportion to the amount of lead ingested[36].

Bhattacharjee et al.[37]. show that exposure to high dose Mn induces oxidative damage leading to altered activity of the enzymes SOD, CAT, GPx, and expression of Mn-SOD and GPx genes.

It is suggested that the first step in the production of ROS is the production of O_2 which can be converted to H_2O_2 by Mn and Cu/Zn superoxide dismutase in the mitochondria and cytoplasm. H_2O_2 can be further converted to OH. in the presence of Mn or other transition metals[38,39].

The Mn^{2+} interferes with the homeostasis of Ca^{2+} in the mitochondria by occupying the Ca^{2+} binding sites with the generation of oxidative stress, leading to the induction of a process called mitochondrial permeability transition. The opening of a permeability transition port leads to the solubility of the mitochondrial membrane for ions and protons which cause the rapid swelling and ultrastructure change associated with the loss of mitochondrial potential of the inner membrane, altered oxidative phosphorylation, ATP synthesis[40].

The Effect of Pb and Mn on the structural architecture of the liver

The results obtained after microscopic observation of histological sections made in the liver in rats intoxicated with Pb and/or Mn showed necrosis with inflammatory infiltrate, hepatocytes and lipid vacuoles within the hepatocytes producing foci of steatosis compared to controls. These results are similar to the studies of Jarrar et al.[41] which suggest that subchronic exposure to lead produces liver damage in rats due to disruption of the antioxidant defense system by free radical generation and oxidative stress, they revealed that lead toxicity leads to free radical damage by two distinct pathways: (1) the generation of ROS, including hydroperoxides, singlet oxygen and hydrogen peroxide and (2) the direct depletion of antioxidant reserves. These results indicate that the hepatic effects of lead were manifested by abnormal serum enzyme levels. The appearance of inflammatory cells in liver tissue due to sub-chronic lead exposure may suggest that lead may interact with proteins and enzymes in the liver interstitial tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) which in turn may mimic an inflammatory response[42].

In fact, the liver is the critical organ for the regulation of Mn homeostasis in the body. Furthermore, Mn toxicity is rare and manifests itself primarily in the liver, probably due to the primary role that this organ plays in eliminating Mn from the blood through biliary excretion[29], consumption of Mn-contaminated water causes the liver to overflow, which can lead to liver damage[30].

After administration of *Cinnamomum cassia* essential oil by intraperitoneal (IP) voice to rats co-exposed to Pb-Mn, a clear improvement in the various biochemical parameters of the intoxicated rats treated with aromatherapy compared to the intoxicated rats was observed. This is due to the different therapeutic and prophylactic virtues of OECC.

However, Chou et al. [34] showed that *C.cassia* EO and cinnamaldehyde, which is its major constituent, have the ability to repress oxidative stress and lipid peroxidation as they interfere with each other by improving the activity of cellular GSH, CAT and GPx, which allows to reduce the rate of ROS. However, Liao et al[43] reported that cinnamaldehyde is the component responsible for modifying the level of antioxidant enzymes resulting from oxidative stress. These results may demonstrate that CCEO may have beneficial effects on certain health problems including their antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic powers. On the other hand, they may even reduce the risk of cardiovascular and neurological diseases [44]and metabolic disorders[45].

According to our results, histological examination of the livers of the intoxicated rats treated showed a hepatic parenchyma remodelled by hepatic steatosis made up of hepatocyte cells containing lipid inclusions in their cytoplasm. Studies suggest that cinnamaldehyde, which is the major component of *C.cassia,* has effects. The anti-inflammatoires mechanism of cinnamaldehyde may be related to ROS and is associated with increased activity of antioxidant enzymes (CAT and GPx). Cinnamaldehyde can be used as a pharmacological agent in the prevention or treatment of a disease in which free radical formation in a pathogenic factor occurs [43].

Table 1: Concentration in % and retention time of the different compounds obtained by gas chromatographic analysis of the essential oil of *Cinnamomum cassia.*

Compound	Retention time (min)	Concentration (%)
E-cinnamaldehyde	22.26	85.77
Linalol	15.10	3.70
Z-cinnamaldehyde	22.60	3.22
p-cymene	06.68	1.71
β-Phellandrene	6.121	1.19
Limonene	9.81	1.189
Eugenol	24.01	0.48
α pinene	10.70	0.395
Terpiene-4-ol	16.70	0.37
Chavical	21.77	0.30
γ-terpinene	10.10	0.291
Camphene	12.55	0.184
β-pinene	10.80	0.109
α -Phellandrene	7.29	0.080

Table 2: Evaluation of body weight parameters of control rats, Pb-Mn, and treated with *C.cassia* essential oil

	Organ	C	Pb-Mn	Pb-Mn+CCEO
Body weight (g)		95,03 ±1,70	67,88±1,35*	76,17 ±1,28
Relative weight (g)	Liver	8,63±0,4	6,33±0,9*	6,53±0,2

The values are expressed in mean ± SEM (*: p<0.05).

Table 3: Effect of *C.cassia* essential oil on different biochemical parameters in Pb-Mn intoxicated rats compared to control rats.

	C	Pb-Mn	Pb-Mn+CCEO
Pb (µg/g)	0,15 ± 0,08	7,02 ± 0,05*	3,4 ± 0,09*
Mn (µg/g)	84,6 ±1,08	118,6 ± 0,71*	89,3 ± 1,01*
ALT (UI/l)	22,11±0,15	48,14± 0,35*	29,50±0,46
AST (UI/l)	14,70±0,20	45,97±0,19*	22,63±0,16*
Total Bilturbine (mg/dl)	0,87 ±0,04	1,17 ±0,01**	0,90±0,02**
Alkaline phosphatase (IU /l)	44,39 ±0,20	97,83 ±0,21*	49,66±0,32*

The values are expressed in average ± SEM **: p<0.01,*: p<0.05).

Table 4: Effect of essential oil of *C.cassia* in the activity of anti-oxidant enzymes in liver of rats intoxicated.

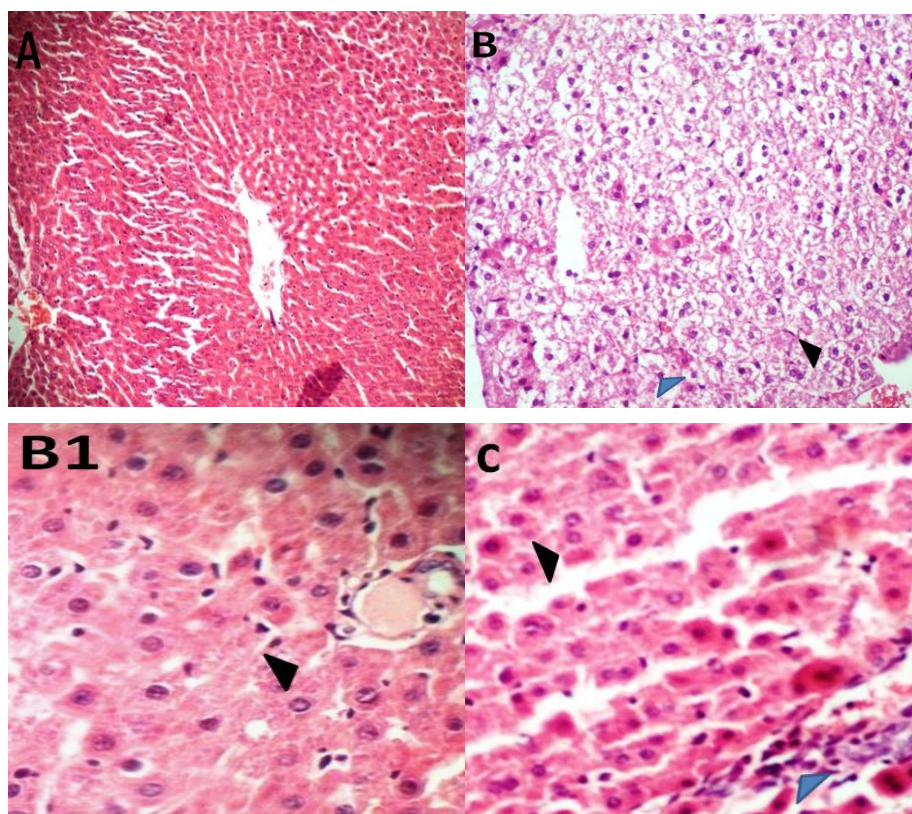| Groups	C	Pb-Mn	Pb-Mn+CCEO
SOD (U/mg)	0,71±0.059	1,19±0,03*	0,89 ±0,019*
CAT (U/mg)	0,39 ±0,34	0,75 ± 0,15*	0,55 ±0,04*
GPx (Um/g)	0,83± 0,07	1,38± 0,23*	0,97± 0,04*

The values are expressed in mean ± SEM (*: p<0.05).

Figure 1: Optical microscopy of liver tissue stained with hematoxylin and eosin G:(x 40). (A)G:(x 100)in sections of the livers of control rats appeared with normal architecture.(B) Rats co-exposed to Pb-Mn showed hepatic necrosis (black arrow), steatosis (blue arrow).(B1) Rats exposed to Pb-Mn showed portal inflammation (black arrow).(C) Rats exposed to Pb-Mn and treated with C. cassia EO showed regenerating hepatocytes (black arrow), portal inflammation (blue arrow).

CONCLUSION

Exposure of Wistar rats during gestation and lactation to Pb-Mn revealed hepatotoxic effects resulting in a significant alteration of the antiradical system represented by the various enzymes. Treatment with OECC in previously intoxicated rats showed a significant improvement in the histopathological image of the liver as well as liver function.

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