Therapeutic effect of *Cinnamomum cassia* essential oil against Mncl2-induced nephrotoxicity in developing wistar rats

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Abstract

The present study aimed to evaluate the protective effect of *Cinnamomum cassia* essential oil on nephrotoxicity induced by manganese chloride (4.79mg/ml) in young Wistar rats during the development period. Wistar rat animals were exposed to manganese through maternal watering on postnatal day (PND) 1 to (PND) 21. After weaning, the rats exposed to manganese received injections of *Cinnamomum cassia* essential oil (0.1 ml/kg) for 21 days. The concentration of manganese in the renal tissues was significantly increased compared to control rats. However, the levels of renal markers such as urea, uric acid, creatinine were significantly increased in the blood after manganese chloride administration. Manganese-induced oxidative stress in renal tissue was indicated by decreased levels of superoxide dismutase, catalase and glutathione peroxidase respectively.

Histologically, the kidney showed several tissue alterations such as necrosis of the glomeruli and tubules. The administration of *Cinnamomum cassia* essential oil considerably attenuated the previous biochemical damage in serum and kidney tissue as well as histological and cellular modifications. In this study, it can be concluded that the *Cinnamomum cassia* essential oil showed a nephro-protective effect.

Keywords: *Manganese chloride, Nephrotoxicity, Cinnamomum cassia, oxidative stress, GC/MS*

Introduction

The contamination of toxic trace elements in the terrestrial and aquatic environments is currently one of the main problems, and increased exposure to these trace elements can lead to undesirable consequences for human health (Junji et. al. 2013).

Indeed, Manganese (Mn) is a natural trace element, essential for the optimal functions of the various organs. It participates in numerous enzymatic reactions such as hydrolase, kinases, decarboxylases and transferases. In addition, it acts as a cofactor for a series of enzymes involved in the metabolism of lipids and carbohydrates but once the recommended dose is exceeded, manganese has been shown to be toxic at several levels, mainly absorbed by the digestive tract and respiratory tract and transported by transporters such as transferrin and albumin to the brain being the first target (Amany et. al. 2015).

However, produced as secondary metabolites by plants, essential oils are involved in several fields, they have found their place in aromatherapy, pharmacy, cosmetics and food preservation (Teixeira et. al. 2013). For decades, *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 (Hamidpour et. al. 2015).

In the light of this information, the aim of our study is to investigate the effect of the essential oil of *Cinnamomum cassia* bark in rats intoxicated by manganese chloride at the renal level in wistar rats during the development period (gestation and lactation).

Materials and Methods

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

The cinnamon bark "*Cinnamomum cassia*" was imported from Sri Lanka, then identified by taxonomic experts (Pr sitayeb). The sample was preserved, 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 injectable solution based on CCEO

A dose of 0.1 ml/kg of *Cinnamomum cassia* essential oil (Li et. al. 2013) was diluted in sterile bidistilled water (100 µL) with tween 80 (Halder et. al. 2011).

Preparation of the oral solution

Manganese chloride tetrahydrate (MnCl2 4H2O) is solubilized in bidistilled water at a dose of 4.79mg/ml (Molina et. al. 2011).

Lot distribution

At day 0 of the coupling the rats are divided into two lots:

Lot Mn: represents rats exposed to manganese ($Mncl_2$) ($C_4H_6Pb_2H_2O$) which receive it orally in bi-distilled water from the first day of the coupling until weaning (Brahmi et. al. 2020).

Lot C (controls): consisting of rats receiving bidistilled water

At day 21 after giving birth, the rats are devised in 3 lots:

Lot Mn: consisting of young pups from the first mating which receive manganese

Lot Mn treated by CCEO: consisting of young pups from the same mating which receive MnCl2 in bidistilled water which is administered intraperitoneally for 21 days after weaning. oride in bidistilled water.

Lot C: consisting of young rats from the same mating that receive drinking water.

Body and kidney weight

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

Biochemical tests

Manganese analysis in tissues of kidney

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 organs

by atomic absorption spectrophotometry (SHIMA DZU AA6200) and the values were expressed in µg /g.

Determination of kidney parameters

Serum concentrations of urea, uric acid (Kaplan, 1984) and creatinine (Murray, 1984) were determined colorimetrically as measures of kidney functions.

Determination of the activity of antioxidant enzymes

The rat kidney was weighed and homogenised in a buffer solution containing 0.32 M sucrose, 0.5 mnM EDTA, 10mM Tris-HCl (pH 7.4) in ice (1mg tissue /4 ml buffer solution) using a glass/glass homogeniser. Tissues were maintained at 4°C during all dissection and homogenisation procedures. The homogenate was centrifuged at 1000xg for 15 minutes at 4°C. The supernatant was then the pellet constitutes the mitochondrial fraction and the supernatant is re-centrifuged at 10,000g/30 minutes. The two 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 the mitochondria trapped in the synaptosomes and centrifuged a second time at 10000×g for 15 min at 4°C, the pellet thus obtained constitutes the total fraction of mitochondria which will be solubilised in a solution containing sucrose (0.32 M at pH 7.4) (Jollow et. al. 1973) Superoxide dismutase (SOD) (EC 1.15.1.1) was analysed on the supernatant using the technique of Wenqiang et. al. (2006); this method is based on the inhibition of the formation of adenine nicotinamide dinucleotide, phenazine methosulphate and aminotetrazolium blue formazan. The activities and levels of renal antioxidants such as catalase (CAT) and glutathione peroxidase (GPx) were analysed by the method of Karousou et. al. (2005); Jollow et. al. (1973), respectively.

Histological study

Samples of Kidney 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 (5 µm thick) were prepared, routinely stained with hematoxylin and eosin (H&E) dyes, and then examined microscopically (Suvarna et. al. 2013; Brahmi et. al. 2020)

Expression and statistical analysis

The results are expressed as the mean (M) of the individual values, affected by the standard error of the mean (SEM). Comparison of multiple means is performed by analysis of variance (one way Anova) with the intoxication factor (Mn, T). Repeated-measures ANOVAs were used for the analysis of the time factor. A probability $p < 0.05$ is considered significative. Statistical analyses were performed with Sigma Stat software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The yield and principal compounds of the essential oil detected by GC/MS

After the hydro-distillation of the plant material of *Cinnamomum cassia*. The yield calculated by the ratio of the weight of the extracted oil to the weight of the plant material used, expressed as a percentage (%) is equal to 1%. this is in agreement with the work of Krishnamoorthy et. al. (1999) and with that of Linsheng et. al. (2013) who reported a yield between (0.4-4.9%).

$$YEO = M'/M.100$$

YEO: yield of essential oil of *Cinnamomum cassia* (%)

M': mass of essential oil obtained in grams (g)

M: mass of *Cinnamomum cassia* used in grams (g)

In addition, the analysis of the essential oil of *Cinnamomum cassia* by gas chromatography coupled to mass spectrometry (GC-MS) permitted the identify of 14 major compounds cited in **Table 01** by order of elution. A total of 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 (85.775%), linalool (3.707%), Z-cinnamaldehyde (3.22%), and B-phellandren (1.19%). these results corroborate with those of Sariözkan et. al. (2016) who show that E-cinnamaldehyde has a percentage of 88.2% in their study.

Table 01. Concentration in % and retention time of the various compounds obtained by gas chromatographic analysis of the essential oil of *Cinnamomum cassia.*

Compounds	Retention time (min)	Concentration (%)
α pinene	10.70	0.395
β-pinene	10.80	0.109
β-Phellandrene	6.121	1.19
α -Phellandrene	7.29	0.080
Camphene	12.55	0.184
Limonene	9.81	1.189
γ-terpinene	10.10	0.291

Chavical	21.77	0.30
Linalol	15.10	3.707
p-cymene	06.68	1.71
Terpiene-4-ol	16.70	0.37
E-cinnamaldehyde	22.26	66.54
Eugenol	24.01	0.48
Z-cinnamaldehyde	22.60	3.22

Impact of manganese and Cinnamomum cassia essential oil (CCEO) on body weight and kidney

Statistical analyses clearly show that the weight of animals exposed to Mn during the prenatal period was significantly ($p<0.05$) decreased compared to the control animals **(Table 02)**, which was manifested by a decrease in body weight gain. This can be explained by the anorexigenic effect of Mn and their impact on the nervous centers responsible for the regulation of satiety and hunger. Our results are in agreement with the work that different authors have undertaken (Torres and Nowson, 2007; Ibrahim et. al. 2012). They observed a reduction in food consumption of intoxicated rats as a function of the dose administered and the duration of exposure. Furthermore, we observed that manganese chloride administration results in a reduction in kidney weight, suggesting a disruption of kidney function and possibly due to the effect of Mn on neuronal proliferation and differentiation during the prenatal period (Smith et. al. 2008).

Table 02. Evaluation of the weight parameters of control, Mn, and *C.cassia* essential oil treated rats

	Organ	**Mn**	**Mn+CCEO**	**C**
Body weight (g)		67,88±1,35*	76,17 ±1,28	95,03 ±1,70
Relative weight (g)	**Kidney**	1,12±0,03*	1,20±0,1	1,49±0,01

Values are expressed as mean ± SEM (*: $p<0.05$).

Consequently, administration of *Cinnamomum cassia* essential oil to rats previously exposed to Mn led to the observation of a marked increase in body weight gain compared to Mn-intoxicated animals. This recorded increase in weight could be due to the presence of terpenoid compounds, which act to stimulate glucose transport in cells (Dwivedi et. al. 2011). Our observations also agree with those of Zargari. (2002), that the addition of medicinal plants stimulates appetite and gastrointestinal fluid secretion, and improves digestion and absorption, thus leading to body weight gain.

Biochemical assay

Determination of manganese levels in kidney tissue

The level of Mn in tissues is an effective biomarker and representative of its exposure. The highest amount of Mn (about 66%), with a half-life of 37 days. (Lee et. al. 2011). The results obtained from renal manganese assays using atomic absorption spectrophotometry (AAS), are significantly ($p<0.05$) increased in rats intoxicated with MnCl2 **(Table 03)**. However, studies have shown that during pregnancy, blood concentrations of Mn increase during the three semesters and Mn enters the placenta by active transport (Krachler et. al. 2002) The increase in Mn levels during pregnancy may also be related to the acceleration of erythropoiesis, intestinal absorption or the tissue that mobilizes Mn (Chowdhury and Das, 1997). Tholin et. al. (1993) also reported that Mn levels during pregnancy increase with each trimester.

Determination of the kidney function parameters

Biochemical analysis of renal biomarkers at the end of the experiment showed changes in plasma levels of renal bioindicators (urea/creatinine) **(Table 03).** This is similar to the experiments of Dorman et. al. (2001), who examined the effects of MnCl2 infusion to normal rats and its interaction with glomerular filtration and renal plasma flow and deduced a significant increase in blood urea and creatinine, due to the antagonistic effect of manganese on calcium ions (Ca+2) during glomerular filtration processes. These results obtained also corroborate with those of Sánchez-González et. al. (2015) found that Mn plays a role in chronic renal failure which causes the increase of urea and creatinine levels. This hypercreatininemia may originate from the accumulation of manganese in the different compartments of the kidney cells.

Table 03. Effect of *C.cassia* essential oil on different biochemical parameters in Mn intoxicated rats compared to control rats.

	Mn	**Mn+CCEO**	**C**
Mn (Kidney) (µg/g)	108± 0,02*	81,2 ±0,1	63±0,10
Urea (mg/dl)	4,70±0,20*	3,97±0,19*	2,63±0,16
Uric acid(mg/dl)	5,11± 0,15*	3,14± 0,35*	2,50±0,46
Creatinine (mg/dl)	10,64±0,15**	8,53± 0,15**	7,20± 0,18

Values are expressed as mean ± SEM **: $p<0.01$, *: $p<0.05$).

Activity of kidney oxidative status enzymes

The evaluation of Mn toxicity was expressed by the determination of antioxidant enzymes such as CAT, GPx , SOD . Considering that these antioxidant enzymes permit to maintain the homeostasis of the redox potential (Chou et. al. 2013). Indeed, the results of the tissue antioxidant status show a significant decrease in the enzymatic activities of catalase (CAT), superoxide dismutase (SOD) and Glutathione peroxidase (GPx), in rats exposed to Mn during the development period compared to control rats **(Table 04)**, leading to a dysfunction of the antioxidant defense system. Bhattacharjee et. al. (2013) show that high dose exposure to Mn induces oxidative damage leading to altered activity of the enzymes SOD, CAT, GPx isoforms, and gene expression of Mn-SOD and GPx.

Table 04. Effect of essential oil of *C.cassia* in the activity of anti-oxidant enzymes in kidney of rats intoxicated

Groups	Mn	Mn+CCEO	C
SOD (U/g)	0,61±0.059*	0,89 ±0,019*	1,15±0,03
CAT (U/g)	0,39 ±0,34*	0,55 ±0,04*	0,75 ± 0,15
GPx (U/g)	0,83± 0,07**	0,97± 0,04**	1,38± 0,23

Values are expressed as mean ± SEM (**: $p<0.01$, *: $p<0.05$).

It is suggested that the first step in ROS production is the production of O2-- which can be converted to H2O2 by Mn and Cu/Zn superoxide dismutase in the mitochondria and cytoplasm. H2O2 can be further converted to OH. in the presence of Mn or other transition metals (Goldstein et. al.1993; Martinez-Finley et. al. 2013).

Mn2+ interferes with Ca2+ homeostasis in mitochondria by occupying Ca2+binding sites with the generation of oxidative stress, which leads to the induction of a process termed mitochondrial permeability transition. The opening of a permeability transition port leads to the solubility of the mitochondrial membrane for ions and protons that cause the rapid swelling and ultrastructure change associated with the loss of mitochondrial inner membrane potential, impaired oxidative phosphorylation, ATP synthesis (Farina et. al. 2012).

Impact of Mn on the structural architecture of the kidney

In the present study, our results of the microscopic examination of the histological sections carried out at the level of the kidneys of the rats intoxicated by Mn showed a necrosis of the glomeruli apparent to their architecture, with a tubular dilatation, tubular vacuolization and an alteration of the tubules on the other hand the control rats presented a normal renal parenchyma comprising renal glomeruli and bypassed tubules, the whole in an interstitial tissue without anomaly **(Fig.01).**

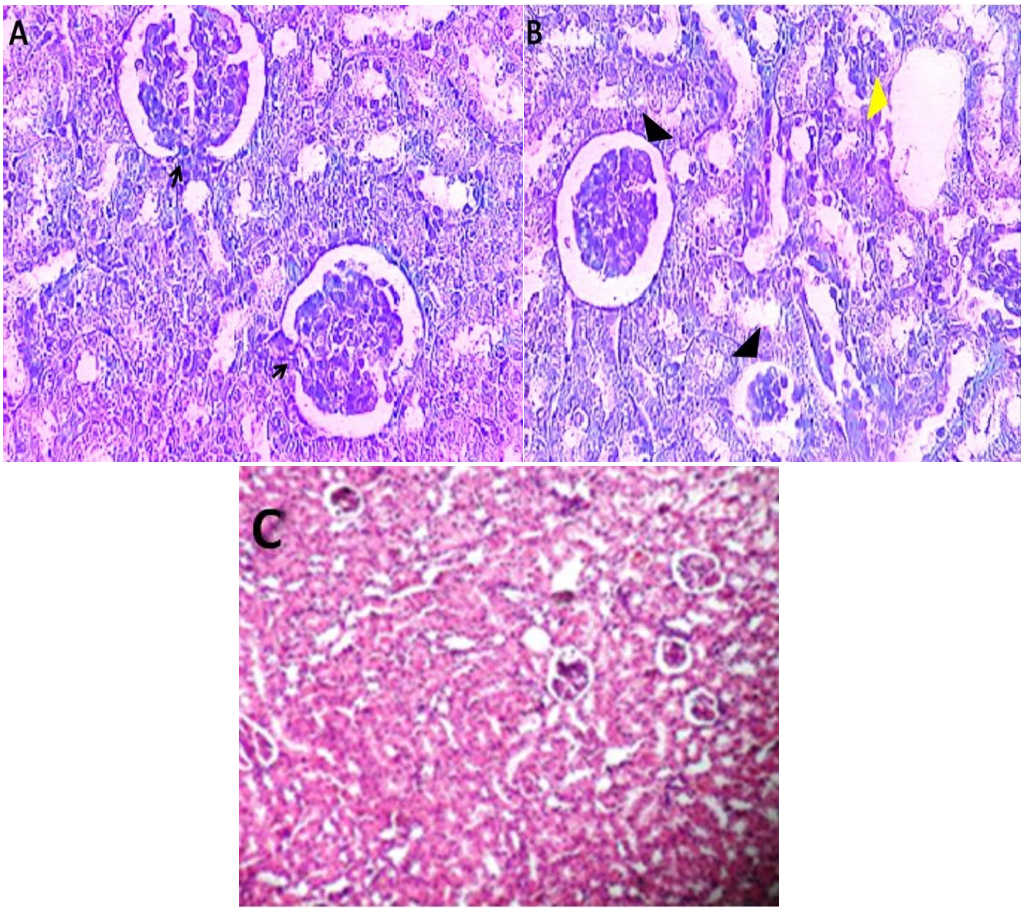

Figure 1. Section of renal rat tissue with hematoxylin and eosin (G:x20); **(A)** normal architecture and normal Bowman capsule in the control group (C) (Animals received distilled water); **(B)** group Mn (Intoxicated animals) Showing degeneration of Bowman capsules, with large interstitial spaces and complete absence of bypassed tubes; **(C)** the group exposed and treated with essential oil (Mn-CCEO), Showing the regeneration of the majority of Bowman's capsules and interstitial cells, with convoluted tubes

Exposure to manganese chloride causes changes in renal architecture (tubular necrosis). This damage invokes the hypothesis of a state of oxidative stress caused by manganese in the cells (Guilherme et. al. 2015). Knowing that manganese can be substituted for different trace elements of the same valence in the metalloproteins (enzymes) that require the presence of these

trace elements in their activities and subsequently induce disturbances in the different metabolisms. Ponapakkam et. al. (2003), reported in their histological study that the most striking lesions were observed in the kidneys of animals.

Impact of Cinnamomum cassia essential oil (CCEO) on biochemical and histological parameters

After the administration of *Cinnamomum cassia* essential oil by intraperitoneal (IP) to rats exposed to manganese chloride, a clear improvement of the different biochemical parameters of the animals treated with aromatherapy was noticed compared to the intoxicated rats. This is due to the different therapeutic and prophylactic virtues of CCEO. It also acts against nephrotoxicity and maintains the biochemical parameters of the kidney at a normal level. It also restores the kidney tissues. It is evident that *Cinnamomum cassia* has a nephroprotective effect (Kumar et. al. 2014).

However, Chou et. al. (2013) showed that *Cinnamomum cassia* EO and cinnamaldehyde, which is its major constituent, have the ability to suppress oxidative stress and lipid peroxidation as they interfere with each other by enhancing the activity of cellular GSH, CAT and GPx, thus reducing ROS levels. However, Lee et. al. (2001) reported that cinnamaldehyde is the component responsible for modifying the level of antioxidant enzymes resulting from oxidative stress.

Indeed, the study of Kumar et. al. (2014) illustrate that after the use of cinnamon an improvement in the renal tissue is observed, the core material and cytoplasm of the glomerulus and Bowman's capsule were effectively restored.

Conclusion

Exposure of wistar rats during gestation and lactation to Mn revealed nephrotoxic effects that result in significant impairment of the antiradical system represented by different enzymes. Treatment with CCEO in previously intoxicated rats showed a significant improvement of the histopathological image of the kidney as well as the renal function.

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Conflicts of Interest

The authors declare that there are no conflicts of interest related to this article.

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