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The Possible Protective Role of Ginger against Carbon Tetrachloride-Induced Nephrotoxicity in Rats. Histopathological and Morphometric Study

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Abstract Carbon tetrachloride (CCl_4) has long been known as a model toxicant. Several reports have discussed its toxic effects on different organs by inducing oxidative stress and free radical production. A number of antioxidant agents, including herbal extracts, have been reported to reduce CCl_4 induced toxicity. Many studies have reported the beneficial effects of ginger including its antioxidant properties. This work was performed to assess the possible protective role of ginger against CCl_4 induced renal injury in adult male albino rats. A total of 48 adult male rats were divided into 3 groups. Group I served as the control group. Group II received CCl_4 by intraperitoneal injections, twice weekly, for 4 weeks. Group III received CCl_4 (as previously described) and aqueous extract of ginger orally, once daily, for 4 weeks. At the end of the experiment, renal specimens were processed for light and electron microscopic examination. In addition, morphometric analysis was performed on electromicrographs to assess the filtration barrier integrity. Carbon tetrachloride treated rats showed renal corpuscles with shrunken, lobulated, and hypercellular glomeruli, podocyte affection, as well as mesangial cell proliferation. Morphometric analysis demonstrated disordered filtration barrier integrity. The use of ginger prevented most of these structural changes. Exposure to CCl_4 resulted in nephrotoxicity associated with glomerular and tubular alterations in adult male rats. Ginger exhibited a protective effect against CCl_4 induced renal damage.

Keywords Carbon tetrachloride, nephrotoxicity, ginger, rat, glomerular filtration integrity.

Introduction

Carbon tetrachloride is a well-known, potent hepatotoxin (Xiao et al., 2012). Recent studies have discussed its toxic effects on organs other than the liver such as the brain, heart, lung and kidneys. The mechanism of CCl_4 induced toxicity includes production of free radicals, which induce lipid peroxidation, besides the activation of tissue macrophages associated with the production of inflammatory mediators (Mourad et al., 2006; Jayakumar et al., 2008).

The implication of oxidative stress and inflammation in the etiology and progression of many clinical disorders has led to the suggestion that agents with antioxidant and anti-inflammatory properties may have health benefits. Several antioxidant agents, including herbal extracts, antioxidant vitamins and melatonin have been reported to reduce CCl_4-induced toxicity (Donder et al., 1999; Turkdogan et al., 2001; Shaker et al., 2011). Zingiber officinale Roscoe (Zingiberaceae) is one of the most widely used spices worldwide. It has an analgesic and anti-inflammatory properties (Jagetia et al., 2003), and it has been reported as an antioxidant and detoxifying agent against alcohol abuse and bromobenzene intoxication (El-Sharaky et al., 2009; Shati and Elsaid, 2009). In addition, the protective effect of ginger was reported against carbon tetrachloride and 6-mercaptopurin induced hepatic damage (Abd-El Aty and Morgan, 2011; Hamed et al., 2012).

Maintaining the balance between free radicals and antioxidants, besides inhibiting the production of inflammatory mediators may serve as major mechanisms in preventing damage induced by this toxic agent. Hence, the major aim of the present study was to assess the potential protective role of ginger on renal corpuscles of CCl_4-intoxicated adult male rats.

Materials and methods

Chemicals

Carbon tetrachloride solution was purchased from Sigma Co., St. Louis, USA. Ginger tablets were provided by Pharco-pharmaceutical Company, Alexandria, Egypt.

Animals

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Tanta University, Egypt. Anesthetic procedures and handling with animals complied with the ethical guidelines of the Research Ethics Committee. Forty eight adult male albino rats, weighing from 180 to 220 gm were used in this study. Animals were housed under standard conditions of temperature (23 ± 2 ºC) and lighting (12 h light/dark cycles) and were allowed free access to food and drinking water.

Experimental design

Group I (Control group): It included 24 rats that were further subdivided into 3 equal subgroups (8 rats each). Subgroup (i) was kept without treatment throughout the study. Subgroup (ii) received 0.5 ml of olive oil (diluting vehicle for CCl_4) by intraperitoneal injections, twice weekly, for 4 weeks. Subgroup (iii) received 1ml of aqueous extract of ginger (100 mg/kg body weight) by gavage, once daily, for 4 weeks (El-Sharaky et al., 2009).

Group II (CCl_4 treated group): included 12 rats that received intraperitoneal injections of 0.5 ml of CCl_4 diluted 1:1 (v/v) in olive oil, twice weekly, for 4 weeks (Marsillach et al., 2009).

Group III (CCl_4 & Ginger treated group): included 12 rats that received CCl_4 and ginger as previously described.

By the end of the experiment, the animals were anesthetized by ether and sacrificed by decapitation and a median abdominal incision was made to deliver the kidneys.

Histopathological examination

For light microscopic study, specimens were taken from the upper pole of the right kidney and were fixed in 10% formalin for preparation of paraffin blocks. Five μm sections were cut and stained by hematoxylin and eosin (H&E) (Gamble, 2008). For electron microscopic examination, (1 mm^3) renal cortical pieces were immediately fixed in 2.5% phosphate buffered gluteraldehyde (pH 7.4) at 4° C for 24 hours and post fixed in 1% osmium tetraoxide for one hour, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Ultrathin sections (80-90 nm) were stained with uranyl acetate and lead citrate, and were examined and photographed with a JEOL transmission electron microscope in EM Unit, Faculty of Medicine, Tanta University (Woods and Stirling, 2008).

Morphometric study and statistical analysis

Morphometric analysis was performed to evaluate four criteria for filtration barrier integrity; the thickness of glomerular basement membrane (BM), foot process base width, slit pore diameter and number of slit pores. This was done in EM unit (Faculty of Medicine, Tanta University) on the TEM images of 80 kV to analyze the number of slit pores per 100 µm of glomerular BM and also to measure the slit pore diameter, the BM thickness and foot process base width at the glomerular BM. Five measurements were performed for each variable per image for each ultrathin section of each group (Whaley-Connell et al., 2006). Values were represented as means and standard deviations. As regards control subgroups, their means and standard deviations were represented in one value of the control group. Statistical analysis was performed using Analysis of variance (ANOVA) test, where the results were considered statistically significant if $p<0.05$.

Results

I- Light microscopy

Light microscopic examination of H&E stained sections of renal cortex of control group shows renal corpuscles having glomeruli with normal cellularity, which are surrounded by capsular spaces and Bowman's capsule. Renal corpuscles are surrounded by many proximal convoluted tubules (PCT) lined by pyramidal cells, and distal convoluted tubules (DCT) lined by cubical cells. These cells have acidophilic cytoplasm and basophilic nuclei (Fig. 1).

Examination of renal cortical sections from CCl_4 treated rats reveals several injured Malpighian corpuscles, which display shrunken, deeply stained glomeruli with widening of the capsular spaces. The surrounding tubules appear dilated with irregular walls and shedding of the lining epithelium, besides pyknosis of their nuclei. The PCT brush borders are lost, with disruption of the tubular walls (Fig. 2).

Sections of renal cortex of the CCl_4 and ginger treated group show almost normal renal architecture. Most of the Malpighian corpuscles and the surrounding PCT and DCT have the same features of the control group (Fig. 3).

II- Electron microscopy

Electron microscopic examination of ultrathin sections of renal cortex of control group reveals podocytes with central nuclei and characteristic primary processes and secondary small foot processes, which are seen regularly spaced and resting on the glomerular basement membrane of blood capillaries. These processes are separated from each other by filtration slits and covered by slit diaphragm. The basal lamina show regular thickness. Mesangial cells are characterized by kidney shaped nuclei surrounded by electron dense cytoplasm and embedded in the thick mesangial matrix. These cells are seen supporting the wall of the glomeruli where podocyte foot processes are absent (Fig. 4).

Ultrathin sections of CCl_4 treated rats show podocytes with disrupted plasma membrane and cytoplasmic rarefaction, together with few normal regularly spaced secondary foot processes. Some foot processes are swollen, disrupted or completely lost. Major foot processes display vacuoles. The glomerular basal lamina show irregular thickening. As well, increase in the number of glomerular mesangial cells is observed (Fig. 5).

Regarding ginger and CCl_4 treated group, their ultrathin sections disclose podocytes with normal euchromatic nuclei and uniform width of basal lamina of capillary endothelium. It is enveloped with many regularly spaced normal looking foot processes (Fig.6).

III- Morphometric analysis

Indicators of glomerular filtration barrier integrity were significantly influenced in the CCl_4 treated group as compared to the control group. Basement membrane thickness, podocyte foot process base width and slit pore diameter were significantly increased in rats treated with CCl_4, but animals treated with both ginger and CCl_4 showed insignificant difference compared to those of the control group. The number of slit pores per 100 um of BM was significantly decreased in CCl_4 treated group, yet the group treated with ginger and CCl_4 showed normalization in this parameter (Table 1).

Table 1. ANOVA one way statistical analysis of the basement membrane thickness, foot processes base width, slit pore diameter and number of slit pores per 100 um of the basement membrane in the studied groups.

	Control group	CCl_4 group	CCl_4 & Ginger group	f	p	p1	p2
BM thickness (um)	0.324±0.056	0.651±0.369	0.379±0.081	4.512	0.001*	0.009*	0.063
Foot processes base width (um)	0.238±0.041	0.442±0.283	0.268±0.042	3.128	0.019*	0.014*	0.071
Slit pore diameter (um)	0.017±0.012	0.062±0.030	0.025±0.021	4.329	0.007*	0.006*	0.078
Number of slit pores/100 um of BM	416.666±146.391	216.666±113.689	316.666±171.241	5.644	0.006*	0.001*	0.059

*CCl_4: carbon tetrachloride, BM: basement membrane, p1: comparison between the control group and CCl_4 treated group, p2: comparison between the control group and CCl_4 & ginger treated group, *significant at $p<0.05$*

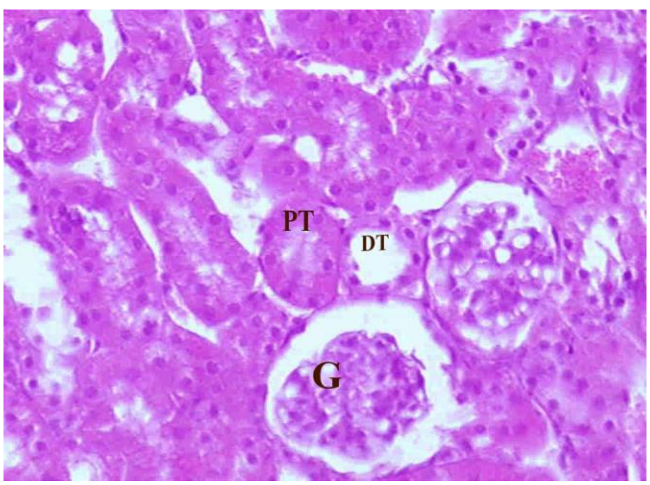

Fig. 1: Photomicrograph of a renal cortex of the control group showing normal looking Malpighian corpuscles (G). Notice proximal (PT) and distal convoluted tubules (DT).
(H&E, Mic. Mag. X 400)

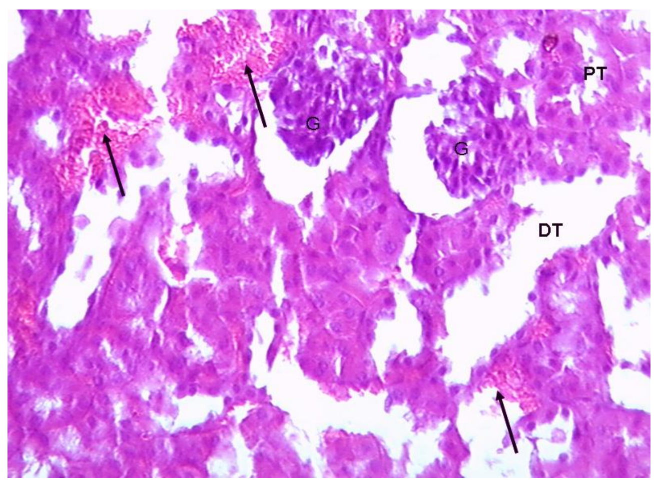

Fig. 2: Photomicrograph of a renal cortex of the CCl_4 treated group showing loss of architecture with interstitial hemorrhage (→). Malpighian corpuscles display shrunken glomeruli (G) with widening of the capsular space. The surrounding proximal (PT) and distal (DT) tubules are dilated with irregular wall and shredded epithelium in association with pyknosis of their nuclei. (H&E, Mic. Mag. X 400)

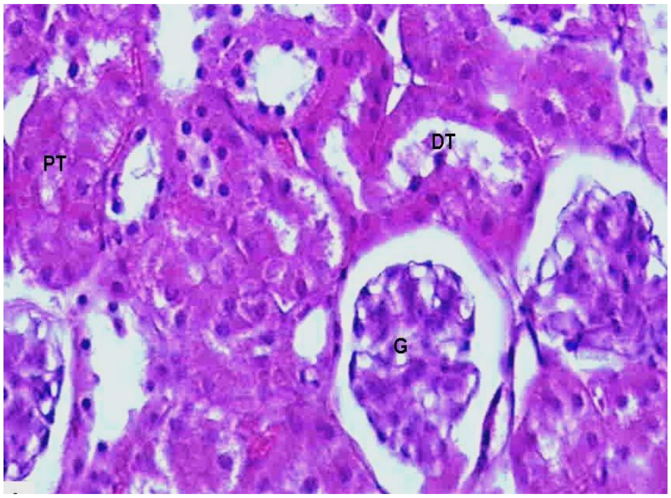

Fig. 3: Photomicrograph of a renal cortex of the CCl_4 and ginger treated group showing normal looking Malpighian corpuscles (G) and normal proximal (PT) and distal (DT) tubules.
(H&E, Mic. Mag. X 400)

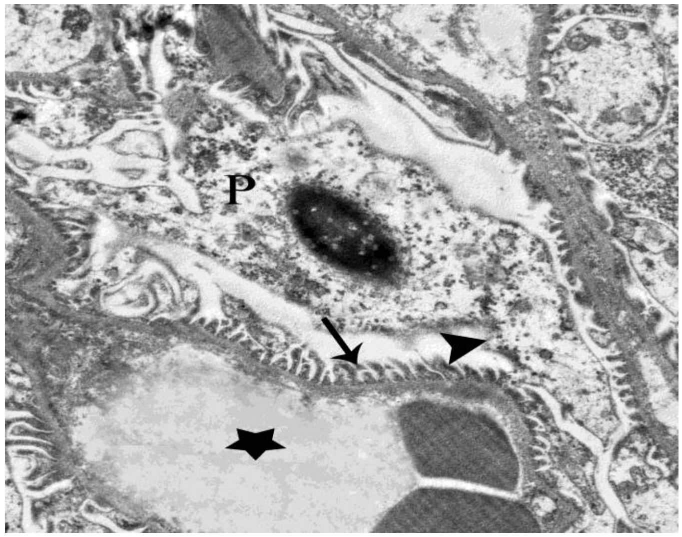

Fig. 4: Electron micrograph of ultrathin section of renal cortex of control group showing podocyte (P) with central nucleus and characteristic well-formed primary process (►) and secondary small foot processes (→) resting on the glomerular basement membrane of blood capillary (*). The basal lamina shows regular thickness. (Mic. Mag. X 2500)

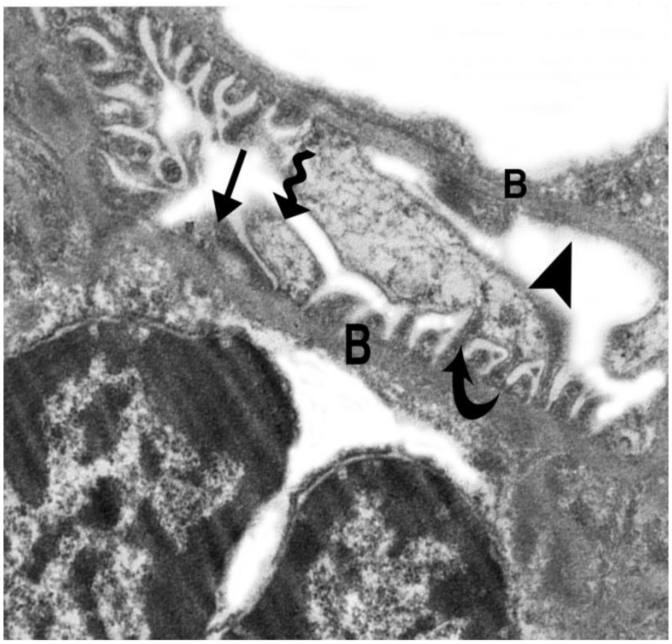

Fig. 5: Electron micrograph of ultrathin section of renal cortex of CCl_4 treated group showing glomerular basement membrane (B) with few regularly spaced secondary foot processes (curved arrow). Secondary foot processes of the podocytes are absent in some areas (►) or effaced (→) or swollen (wavy arrow) in other areas. The basal lamina shows irregular thickening.
(Mic. Mag. X 5000)

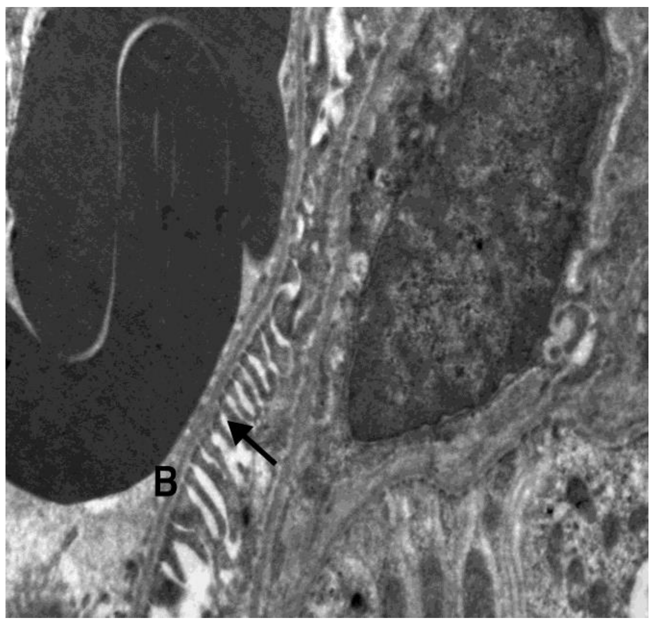

Fig. 6: Electron micrograph of ultrathin section of renal cortex of CCl_4 and ginger treated group showing podocyte with normal nucleus, uniform width of basal lamina of capillary endothelium (B), which is enveloped with many regularly spaced normal looking foot processes (→).
(Mic. Mag. X 3000)

Discussion

The present work revealed that CCl_4 induced manifest structural and ultrastructural changes associated with disordered glomerular filtration barrier integrity in the renal corpuscles of adult male rats. Shrunken hypercellular glomeruli with thickening of the glomerular basement membrane and podocyte ultrastructural changes were all evident. Mostly these changes were prevented when animals were treated with ginger along with CCl_4.

Glomerular sclerosis found in the present study was reported also by other researchers (Doğukan et al., 2003; Ganie et al., 2011). Similar findings associated with significant alterations in antioxidant enzyme activities were shown, indicating the occurrence of oxidative stress and increase in free radical production (Ozturk et al., 2003). Carbon tetrachloride induced nephrotoxicity was assumed to be secondary to liver impairment, as cirrhotic rats with CCl_4 induced liver damage exhibited manifest renal structural changes associated with significant reduction of renal functions (Rincón et al., 1999). However, renal damage might develop independently of hepatic involvement, with oxidative stress and generation of reactive oxygen species (ROS) as one of the suggested mechanisms for CCl_4 induced nephrotoxicity. Carbon tetrachloride was shown to enhance the generation of ROS, which may provoke membrane lipid peroxidation of the endoplasmic reticulum, leading to disturbed Ca^{2+} homeostasis and finally cell death. This could explain the atrophied glomeruli and disrupted plasmalemma of the parietal layer of Bowman's capsule and of podocytes observed in this study (Duque et al., 1992; Abraham et al., 1999; Adewole et al., 2007).

Glomerular collapse and widening of the capsular space reported in the current study may result from contraction of mesangial cells that is usually coupled with contraction of the basement membrane of glomerular capillary endothelium. Glomerular sclerosis may possibly be related to vasoconstriction secondary to the induction of oxidative stress and the subsequent release of vasoactive mediators. In addition, increased collagen production by mesangial cells plays a key role in the development and progression of glomerular sclerosis (Garcia-Cohen et al., 2000; Hamed et al., 2012).

Examination of the renal cortex of CCl_4 treated rats showed hypercellular glomeruli, which was reported also by other studies (Rincón et al., 1999; Jaramillo-Juárez et al., 2008). The current study also revealed thickening of the basement membrane that was confirmed by morphometric analysis. This could be explained in light of the increased release of ROS, which provokes proliferation of mesangial cells. These cells are the major contributor of the extracellular matrix, which contains type IV collagen and fibronectins (Adewole et al., 2007; Dentelli et al., 2007).

Corresponding to the results of Ogata et al. (1995) the present study revealed lots of seriously affected podocytes with effacement of their foot processes in the renal cortex of CCl_4 treated rats. Podocyte structural changes may result in retraction of their foot processes, increased permeability of the glomerular filtration barrier and proteinuria along with deterioration of the renal functions (Pavenstädt et al., 2003).

The present study showed normal appearance of most Malpighian corpuscles associated with average filtration barrier integrity parameters in rats treated with both CCl_4 and ginger. Other researchers reported comparable results. The favorable effect of ginger was assumed to be due to its ability to enhance the activities of antioxidant enzymes and to reduce inflammation and improve kidney function. Moreover, ginger is rich in natural antioxidants, and it can clean up free radicals generated by CCl_4 (Stoilova et al., 2007; Hamed et al., 2012).

From the present study, it could be concluded that CCl_4 has a toxic effect on the glomerular filtration barriers of adult male albino rat kidney, which was largely prevented by concomitant administration of ginger.

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الملخص العربي

الدور الوقائي المحتمل للزنجبيل ضد تسمم الكلى الناجم عن رابع كلوريد الكربون في الجرذان: دراسة هستوباثولوجية و مورفومترية

أحمد عبد الستار الإبيارى و أروة أحمد أبوالفضل١ و إيهاب محمد هنطش٢ و أحمد السيد عبدالفتاح٣ وأحمد عاصم عبدالمطلب وصباح حسين الغايش٤

يعتبر رابع كلوريد الكربون منذ زمن طويل نموذجا للتجارب و الدراسات السمية، وقد ناقشت العديد من التقارير آثاره السامة على أجهزة الجسم المختلفة عن طريق حفز الاكسدة وإنتاج الشوارد الحرة، وقد استخدم عدد من المواد المضادة للأكسدة، بما في ذلك بعض مستخلصات الأعشاب، للحد من سميته، و قد أشارت العديد من الدراسات للآثار المفيدة للزنجبيل بما في ذلك خصائصه المضادة للأكسدة.

تم تنفيذ هذا العمل لتقييم الدور الوقائي المحتمل للزنجبيل ضد تسمم الكلى الناجم عن التعرض لرابع كلوريد الكربون في ذكور الجرذان البيضاء.

تم تقسيم ثمانية و أربعين من ذكور الجرذان البيضاء البالغة إلى ثلاث مجموعات، المجموعة الأولى كانت بمثابة المجموعة الضابطة، وتلقت المجموعة الثانية رابع كلوريد الكربون عن طريق الحقن داخل الصفاق مرتين أسبوعيا لمدة أربعة أسابيع، بينما تلقت المجموعة الثالثة رابع كلوريد الكربون (بالطريقة ذاتها) بالإضافة إلى الزنجبيل عن طرق الفم مرة واحدة يوميا لمدة أربعة أسابيع، وفي نهاية التجربة تم تجهيز عينات الكلى لفحصها بواسطة المجهرين الضوئي والإلكتروني، وبالإضافة إلى ذلك تم إجراء قياسات مورفومترية لتقييم معايير سلامة حاجز الترشيح الكلوى.

أظهرت الجسيمات الكلوية للفئران المعالجة برابع كلوريد الكربون انكماشاً وتفصصاً و زيادةً خلوية فى كبيباتها، مع تأثر الخلايا الرجلاء سلبيا، بالإضافة إلى زيادة عدد خلايا مسراق الكبيبة (خلية المسانجيم)، و قد أوضحت القياسات المورفومترية تأثر حاجز الترشيح الكلوى سلبيا، و قد وجد أن استخدام الزنجبيل قد منع معظم هذه التغيرات البنيوية.

أدى التعرض لرابع كلوريد الكربون لتسمم الكلى الذي صاحبه تغيرات في الكبيبات و الأنابيب الكلوية في ذكور الجرذان البالغين، و كان للزنجبيل تأثير وقائي ضد الضرر الكلوي الناجم عن رابع كلوريد الكربون.

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