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Defensive role of *Rosmarinus officinalis* in carbon tetrachloride-induced nephrotoxicity and oxidative stress in rats

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Abstract

Background: There is a growing demand for remedies from natural sources to substitute synthetic therapeutic drugs and minimize their side effects and toxicity. The present study aims to evaluate the defensive ability of an ethanolic extract of *Rosmarinus officinalis* L. in carbon tetrachloride (CCl_4)-induced nephrotoxicity in male albino rats.

Materials and methods: Thirty-six rats were divided into 6 groups ($n = 6$). Group I (control) received distilled water for 30 days orally. Nephrotoxicity was induced by CCl_4 (11% *v/v* with olive oil, i.p) 2 ml/kg body weight (b.wt.) in group II once a week for 30 days. Groups III and IV received the only herb in two doses 100 and 250 mg/kg of b.wt. respectively. Groups V and VI received an ethanolic extract of *Rosmarinus officinalis* (EERO, 100 and 250 mg/kg of b. wt.) along with 2 ml/kg b.wt. CCl_4 weekly for 30 days.

Results: CCl_4 treatment induced highly significant ($P < 0.001$) elevation in kidney biomarkers, i.e., blood urea nitrogen and creatinine, kidney biochemicals, i.e., LPO and XOD, and decrease the levels of superoxide dismutase, catalase, glutathione peroxidase, and glutathione in tissue. However, EERO significantly ($P < 0.001$) restored the altered levels of these biomarkers in a dose-dependent manner. Furthermore, EERO also prevents histological alteration caused due to the toxicity of CCl_4.

Conclusion: Our findings strongly support that ethanolic extract of *Rosmarinus officinalis* acts as a potent scavenger of free radicals to prevent the toxic effect of CCl_4 and hence validate its ethnomedicinal use.

Keywords: Nephrotoxicity, Rat, Carbon tetrachloride, *Rosmarinus officinalis*, Histopathology

Introduction

Nephrotoxicity is one of the most common kidney problems and occurs when the body is overexposed to a drug or toxin (Porter and Bennett 1981). A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis, and nephritic syndrome because there is an increasing number of potent therapeutic drugs like aminoglycoside antibiotics, non-steroidal anti-inflammatory drugs (NSAID's), and chemotherapeutic agents which have been added to the therapeutic arsenal (Hoitsma et al. 1991). Exposure to chemical reagents like ethylene glycol, CCl_4, sodium oxalate, and heavy metals induce nephrotoxicity. Prompt recognition of the disease and termination of responsible drugs are usually the only necessary therapy (Azab et al. 2017).

India is experiencing a rapid health transition with large and rising burdens of chronic diseases, which are estimated to account for 53% of all deaths and 44% of disability (Srinath et al. 2005). According to the World Health Organization (WHO), Global Burden of Disease project, diseases of the kidney and urinary tract contribute globally with approximately 850,000 deaths every year and 115,010,107 disability-adjusted life (Dirks et al. 2006). Chronic kidney diseases (CKD) is the 12th leading cause of death and 17th cause of disability. Treatment of kidney-related diseases is very expensive, relatively unavailable with high incidences of adverse effects and failure (Corsonello et al. 2005). Due to the expensive and complex treatment

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system, very few patients are able to obtain adequate medicinal facility.

Therefore, medicinal plants have been used by all civilizations as a source of medicines since ancient times. In the recent times, there has been growing interest in exploiting the biological activities of different Ayurvedic medicinal herbs, due to their natural origin, cost-effectiveness, and lesser side effects (Naik et al. 2003). Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs and the bioprospecting of new plant-derived drugs (Sharma et al. 2010).

The medicinal plant *Rosmarinus officinalis*, commonly known as rosemary, belongs to family Lamiaceae, has been used as medicinal, culinary, and cosmetics in ancient Egypt, Mesopotamia, China, and India (Stefanovits-Banyai et al. 2003). It is used as carminative, rubefacient, and stimulant and as a flavoring agent for liniments, hair lotions, inhaler, soaps, and cosmetics (Kokate et al. 2001) and as a cholagogue, diaphoretic, digestant, diuretic, emmenagogue, laxative, and tonic (Bedevian 1994; Farnsworth 2005). It is also having potent role in treatment and prevention of diseases like bronchial asthma, spasmogenic disorders, peptic ulcer, inflammatory diseases (Al-Sereiti et al. 1999), hepatotoxicity, atherosclerosis biliary upsets, as well as for tension headache, renal colic, heart disease, and poor sperm motility (Rampart et al. 1986; Al-Sereiti et al. 1999). Phytochemical screening of rosemary revealed the presence of tannins, reducing sugar, flavonoids, alkaloids, carbohydrates, glycosides, and terpenoids (Akram et al. 2016 and Maajida et al. 2017). Carnosol, carnosic acid, methyl carnosate, rosemarinic acid, and isorhamnetin-3-O-hexoside are major components found in *Rosmarinus officinalis* (Amar et al. 2017).

CCl_4-induced nephrotoxic rats have been considered as a good model for evaluation of nephroprotective agents. Carbon tetrachloride, besides exerting its toxic effect on the liver, also reportedly gets distributed at higher concentrations in the kidney than in the liver (Sanzgiri et al. 1997). Various studies have demonstrated that CCl_4 causes free radical generation in many tissues including kidney. Olagunjua et al. (2009) suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl_4 nephrotoxicity. Noguchi et al. (1982) reported that CCl_4 resulted in the enhanced generation of trichloromethyl peroxyl radical and hydrogen peroxide in cultured hepatocytes as well as mesangial cells in the kidney. In addition, a report on various documented case studies established that CCl_4 produces renal diseases in humans (Ruprah et al. 1985). In vitro and in vivo studies indicate that CCl_4 enhances lipid peroxidation, reduces renal microsomal NADPH cytochrome P450, and renal reduced/oxidized glutathione ratio (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria (Rungby and Ernst 1992). However, not much pharmacology data regarding the nephrocurative and antioxidant effect of *Rosmarinus officinalis* is available. So, the present study was designed to evaluate the protective potential of *Rosmarinus officinalis* against carbon tetrachloride-induced nephrotoxicity and oxidative stress in rats.

Materials and methods

Chemicals and reagents

All the chemicals used were of analytical grade obtained from Merck, Mumbai and HiMedia, Mumbai. Blood urea and creatinine investigations were performed using commercially available diagnostic kits of Erba Mannheim, Germany.

Preparation of *Rosmarinus officinalis* extract

An upper shoot of *Rosmarinus officinalis* (along with flowers) was purchased from Indian Institute of integrative medicine, Srinagar J&K, India. The plant material was washed with double-distilled water and thereafter shade-dried for the period of 3 weeks at room temperature. The fully dried plant material was powdered with the help of mechanical grinder. The powder was extracted in 90% ethanol (10% H_2O) by using the Soxhlet extractor. The ethanol extract was then dried under vacuum and the semisolid material thus obtained was stored in storage vials which were kept at − 4 °C for further use. The fresh stock solution of *Rosmarinus officinalis* 80 mg/ml was prepared in double distilled water just before use.

Experimental animals

Wistar albino rats, weighing (235 ± 15 g) were obtained from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, Madhya Pradesh, India. Animals were maintained under standard conditions of temperature 23 ± 1 °C and with regular 12:12 h's light/dark cycle and allowed free access to standard laboratory food (Golden feeds, Delhi) and water ad libitum. All animal experiments were performed as per the guidelines of the committee for the purpose of control and supervision on experiments on animals (CPCSEA Reg. No.- 1283/c/09/CPCSEA). Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Approval No. PBRI/13/IAEC/PN-296a).

Experimental animals

The animals were divided at random into six groups; each group contains six rats, treated as follows. Group I (control) received distilled water for 30 days orally. Group II received carbon tetrachloride (11% *v*/*v* with olive oil) 2 ml/kg b.wt. once a week for 30 days. Groups III and IV received only herb ethanolic extract of *Rosmarinus officinalis* (EERO) at the doses of 100 mg/kg and 250 mg/kg of b.wt. for 30 days respectively. Group

V received EERO orally 100 mg/kg of b.wt. daily followed by a dose of CCl_4 2 ml/kg b.wt. once a week for 30 days. Group VI received 250 mg/kg b.wt. of EERO daily followed by a dose of CCl_4 2 ml/kg b.wt. once a week for 30 days. At the end of the experiment, animals were fasted overnight, blood samples were collected by cardiac puncture, under light diethyl ether anesthesia into previously labeled EDTA retaining tubes and centrifuged in Remi centrifuge at 4 °C for 10 min at 5000 rpm as to get the plasma. The obtained plasma was used for the measurement of kidney markers like blood urea nitrogen (BUN) and creatinine by using commercially available kits. Then animals were dissected out and kidney was excised, washed in freshly prepared ice-cold 0.9% saline to remove blood, and freed from fat. Then homogenate was prepared in phosphate and Tris HCl buffers.

Biochemical parameters

Blood urea nitrogen was determined by GLDH Urease Method, Initial Rate (Talka and Schubert 1965; Tiffany et al. 1972) while creatinine was estimated by Jaffe's method (Bowers 1980; Slot 1965; Young 1975).

Antioxidant assay

The kidneys were minced separately into small pieces and homogenized with ice-cold 0.05 M potassium phosphate buffer and Tris HCl buffer to make 10% homogenates. The homogenates were centrifuged at 4500 rpm for 15 min at 4 °C. The supernatant was collected for estimations of superoxide dismutase (SOD) by Paoletti et al. (1986), catalase (CAT) by Goth (1991), glutathione peroxidase (GPx) by Wendel (1980), glutathione (GSH) by Ellman (1959), xanthine oxidase (XOD) by Bergmeyer et al. 1974), and lipid peroxidation (LPO) by Ohkawa et al. 1979).

Histopathological studies

Portions of each kidney from all the experimental groups were fixed in 10% formaldehyde, dehydrated in graded alcohol, cleared in xylene, and then embedded in paraffin. Microtome sections (5 μm-thick) were prepared from each kidney sample and stained with hematoxylin-eosin dye and processed further as described by (Raghuramulu et al. 1983). The sections thus obtained were then examined for the pathological findings and later on scanned in the microscope (Olympus 'CH20I' Trinocular) at × 40 with photographic facility and photomicrographs were taken using Sony digital camera attached to the microscope.

Statistical analysis

Data were expressed in mean ± SD. Statistical comparison between different groups was done by using one-way ANOVA followed by Bonferroni's test. $P < 0.05$ and $P < 0.001$ were considered as levels of significance.

Results

Effect of ethanolic extract of *Rosmarinus officinalis* on CCl_4-induced changes

The level of blood urea nitrogen (BUN) and creatinine in a control group of rats were 17.99 ± 1.20 mg/dl and 0.88 ± 0.14 mg/dl respectively. Intra-peritoneal administration of CCl_4 2 ml/kg b.w. once a week for 30 days caused abnormal renal function in all experimental animals. BUN and creatinine levels were highly significantly ($P < 0.001$) elevated to 39.62 ± 3.20 mg/dl and 2.08 ± 0.21 mg/dl, i.e., increased by + 120.23% and + 136.36% respectively of their control values.

However, in the animals which received ethanolic extract of *Rosmarinus officinalis* at 100 mg/kg and 250 mg/kg, no significant variations in the levels of BUN and creatinine was noticed and the values of these parameters in EERO 100 mg/kg treated group of rats was BUN 19.47 ± 1.92 mg/dl and 0.97 ± 0.10 mg/dl of creatinine, and the marginal and percentage inhibition for these kidney markers against a control group of rats was (+ 8.22%) and (+ 10.22%) respectively. In a group of rats supplied with EERO 250 mg/kg, the levels of BUN and creatinine was 18.10 ± 1.55 and 0.96 ± 0.21 mg/dl with percentage inhibition (+ 0.61%) and (+ 9.09%) against a control group of rats respectively. Thus, these results revealed that the EERO was not having any type of side effect on kidneys. Hence, the extract was safe at selected doses. Pre-treatment with EERO at 100 mg/kg along with CCl_4 restored the altered levels of BUN and creatinine to 22.19 ± 2.12 mg/dl and 1.07 ± 0.17 mg/dl were highly significant as compared CCl_4-treated group and their percentage inhibition was (+ 23.34%) and (+ 21.59%) respectively, when compared to control group, i.e., the rats which only distilled water for 30 days orally. However, in group VI, i.e., animals which received EERO at 250 mg/kg along with CCl_4, the levels of BUN and creatinine was further reduced by (+ 3.16%) and (+ 15.90%) respectively (Table 1), and the reduction in the levels of BUN and creatinine was highly significant when compared to that of group II. Thus the extract showed a protective effect at both doses, i.e., 100 and 250 mg/kg against carbon tetrachloride-induced nephrotoxicity and the protection was offered in a dose-dependent manner.

Effect of ethanolic extract of *Rosmarinus officinalis* on renal antioxidant profile

We also studied various enzymes in kidney homogenate which are involved in oxidative stress and the findings of our investigation with a control group of rats revealed the levels of SOD, CAT, GSH, GPx, LPO, and XOD as 2.92 ± 0.10 U/mg, 24.49 ± 2.01 U/mg, 533.17 ± 11.94 nM/mg, 5.92 ± 0.19 U/mg, 0.220 ± 0.011 nM/mg, 307.86 ± 27.70 IU/g respectively. However, CCl_4 intoxication significantly decreased the activity levels of SOD to 1.85 ± 0.180 U/mg

Table 1 Effect of ethanolic extract of upper shoot of *Rosmarinus officinalis* (EERO) on BUN, urea, and creatinine in CCl_4-induced nephrotoxicity in rats

Groups	BUN (mg/dl)	Creatinine (mg/dl)
Control	17.99 ± 1.20	0.88 ± 0.14
CCl_4	39.62 ± 3.20 (+ 120.23%)	2.08 ± 0.21 (+ 136.36%)
EERO 100 mg/kg	19.47 ± 1.92 (+ 8.22%)	0.97 ± 0.10 (+ 10.22%)
EERO 250 mg/kg	18.10 ± 1.55 (+ 0.61%)	0.96 ± 0.21 (+ 9.09%)
EERO 100 mg/kg + CCl_4	22.19 ± 2.12** (+ 23.34%)	1.07 ± 0.17** (+ 21.59%)
EERO 250 mg/kg + CCl_4	18.56 ± 1.31** (+ 3.16%)	1.02 ± 0.11** (+ 15.90%)

All data presented in Mean ± SD (n = 6) and **P < 0.001 (highly significant) as compared to CCl_4 group. + = % increase, − = % decrease, all groups were compared with control group which received distilled water for 30 days orally

(− 36.64%), CAT to 12.59 ± 1.91 U/mg (− 48.59%), GSH to 310.80 ± 9.92 nM/mg (− 41.70%), GPx to 3.97 ± 0.27 U/mg (− 32.93%), and elevated the level of LPO and XOD to 0.431 ± 0.042 nM/mg (+ 95.45%) and 615.61 ± 20.19 IU/mg (+ 99.96%) respectively. In group III of rats which received EERO at 100 mg/kg, the activity levels of SOD, CAT, GSH, GPx, LPO, and XOD were near control levels viz. 2.73 ± 0.14 U/mg, 22.51 ± 2.10 U/mg, 526.88 ± 15.23 nM/mg, 5.93 ± 0.09 U/mg, 0.269 ± 0.008 nM/mg, and 321.02 ± 16.70 IU/mg respectively. The percentage inhibition of SOD, CAT, GSH, GPx, LPO, and XOD in 100 mg/kg of EERO-treated group against control group was (− 6.50%), (− 8.08%), (− 1.17%), (+ 0.16%), (+ 18.18%), and (+ 4.28%) respectively when compared with control group. However, in group IV, i.e., the group of rats supplied with EERO at 250 mg/kg, the results observed were in close proximity to control levels viz. SOD 2.71 ± 0.11 U/mg, CAT 26.25 ± 2.27 U/mg, GSH 535.76 ± 11.46 nM/mg, GPx 5.94 ± 0.11 U/mg, LPO 0.257 ± 0.01 nM/mg, and XOD 315.73 ± 21.76 IU/mg with percentage inhibition (− 7.19%), (+ 7.18%), (+ 0.48%), (+ 0.33%), (+ 13.63%), and (+ 2.55%) respectively for SOD, CAT, GSH, GPx, LPO, and XOD. These results clearly indicated the non-toxic nature of EERO on both selected doses (Table 2).

The activity levels of SOD, CAT, GSH, and GPx were significantly elevated, whereas the activity levels of LPO and XOD were significantly reduced in rats treated with both 100 mg/kg of EERO along with CCl_4 (group V) viz. SOD 2.36 ± 0.17 U/mg (− 19.17%), CAT 17.93 ± 2.07 U/mg (− 26.78%), GSH 506.90 ± 15.48 nM/mg (− 4.92%), GPx 5.75 ± 0.19 U/mg (− 2.87%), LPO 0.306 ± 0.013 nM/mg (+ 36.36%), and 351.27 ± 27.12 IU/mg (+ 14.10%). However, further elevation in the activity levels of SOD, CAT, GSH, and GPx was noticed when rats were given access to 250 mg/kg of EERO alongside with CCl_4 (group VI) viz. SOD 2.57 ± 0.11 U/mg (− 11.98%), CAT 21.04 ± 1.85 U/mg (− 14.08%), GSH 520.22 ± 15.10 nM/mg (− 2.42%), GPx 5.86 ± 0.13 U/mg (− 1.01%) but the levels of LPO and XOD were reduced to 0.241 ± 0.011 nM/mg (+ 9.09%) and 325.69 ± 23.72 IU/mg (+ 5.79%) respectively. These results obtained clearly illustrated dose-dependent working potential of ethanolic extract of *Rosmarinus officinalis* (EERO).

Effect of ethanolic extract of *Rosmarinus officinalis* on renal histopathology

The biochemical results of our investigations were fully assured by histopathological examinations of kidney micro sections. The histological inspection of the kidney of control rats (group I) revealed that the cortex consists of several Bowman's capsule which are double layered cup-like structure inside highly anatomizing set of connections of afferent and efferent arterioles called glomerulus were present. The cortical tubules were well structured with connective tissue and inter tubular spaces. Tubular walls were made up of thick epithelial cells. Urinary space and vascular pole were well defined.

Table 2 Effect of ethanolic extract of *Rosmarinus officinalis* (EERO) on, SOD, CAT, GSH, GPx, LPO, and XOD in CCl_4-induced nephrotoxicity in rats

Groups	SOD (U/mg)	CAT (U/mg)	GSH (nM/mg)	GPx (U/mg)	XOD (IU/gm)	LPO (nM/mg)
Control	2.92 ± 0.10	24.49 ± 2.01	533.17 ± 11.94	5.92 ± 0.19	307.86 ± 27.70	0.22 ± 0.011
CCl_4	1.85 ± 0.18 (− 36.64%)	12.59 ± 1.91 (− 48.59%)	310.80 ± 9.92 (− 41.70%)	3.97 ± 0.27 (− 32.93%)	615.61 ± 20.19 (+ 99.96%)	0.43 ± 0.04 (+ 95.45%)
EERO 100 mg/kg	2.73 ± 0.14 (− 6.50%)	22.51 ± 2.10 (− 8.08%)	526.88 ± 15.23 (− 1.17%)	5.93 ± 0.09 (+ 0.16%)	321.02 ± 16.70 (+ 4.28%)	0.26 ± 0.01 (+ 18.18%)
EERO 250 mg/kg	2.71 ± 0.11 (− 7.19%)	26.25 ± 2.27 (+ 7.18%)	535.76 ± 11.46 (+ 0.48%)	5.94 ± 0.11 (+ 0.33%)	315.73 ± 21.76 (+ 2.55%)	0.25 ± 0.01 (+ 13.63%)
EERO 100 mg/kg + CCl_4	2.36 ± 0.17** (− 19.17%)	17.93 ± 2.07* (− 26.78%)	506.90 ± 15.48** (− 4.92%)	5.75 ± 0.19** (− 2.87%)	351.27 ± 27.12** (+ 14.10%)	0.30 ± 0.013** (+ 36.36%)
EERO 250 mg/kg + CCl_4	2.57 ± 0.11** (− 11.98%)	21.04 ± 1.85** (− 14.08%)	520.22 ± 15.10** (− 2.42%)	5.86 ± 0.13** (− 1.01%)	325.69 ± 23.72** (+ 5.79%)	0.24 ± 0.01** (+ 9.09%)

All data presented in Mean ± SD (n = 6) and *P < 0.05 (significant) **P < 0.001 (highly significant) as compared to control group. + = % increase, −=% decrease, all groups were compared with control group which received distilled water for 30 days orally

On the one hand, CCl_4-inebriated rats (group II) showed glomerular hypertrophy, degeneration of epithelial layer of Bowman's capsule, prominent loss of urinary space between glomerulus and Bowman's capsule, loss of brush border in proximal tubules, inflammatory cell infiltrations, cast formation in renal tubules, moderate to severe necrosis of tubular epithelium, congestion and dilation of blood vessels. However, the treatments of EERO at 100 mg/kg and 250 mg/kg, i.e., groups III and IV, showed the similar structural design of micro sections of the kidney as that of a control group of rats. On the other hand, when CCl_4 intoxicated rats were supplied with EERO 100 mg/kg (group V), the kidney sections showed normal architecture but still, slight hypercellularity was observed in the glomerulus. However, in the case of group VI which received EERO at 250 mg/kg along with CCl_4, normal structure of kidney was witnessed as evident from well-defined Bowman's capsule with glomerulus, distinct urinary space, and normal proximal and distal convoluted tubules.

Photomicrographs of T.S. of the kidney—Fig. 1 (group I)—control group of rats showing normal architecture with well-defined Bowman's capsule (BW) with glomerulus(G), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), urinary space (US), and vascular pole (VP) (× 40, haematoxylin-eosin stain). Figure 2 shows photomicrographs of the kidney of rats inebriated with CCl_4 (2 ml/kg with 50% olive oil, weekly for 30 days). Figures 3 and 4 show the kidney section of only EERO-treated rats at a dose of 100 mg/kg and 250 mg/kg of body weight. Figure 5 shows the photomicrographs of the kidney of rats treated with a daily dose of EERO 100 mg/kg and CCl_4 once a week. Figure 6 shows the photomicrographs of the kidney of rats treated with a daily dose of 250 mg/kg of EERO and CCl_4 once a week.

Discussion

Carbon tetrachloride, besides exerting its toxic effect on the liver, also reportedly gets distributed at higher concentrations in the kidney than in the liver (Sanzgiri et al. 1997). The mechanism of CCl_4-renal toxicity is almost same as that of the liver, but CCl_4 shows a high affinity to the kidney cortex which contains cytochrome P-450 predominantly (Abraham et al. 1999; Jaramillo-juarez et al. 2008). Cumulative data suggest a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl_4 nephrotoxicity (Recknagel et al. 1989). Kidneys have some fragile responsibilities, especially when they have to deal with unwanted substances, which they have to clear from the system, especially toxins. Kidney toxicity caused a rapid decline in renal functions that is mainly attributed to decrease in glomerular filtration rate (GFR) and lack of ability of the kidney to excrete these toxic metabolites produced in our body resulting in abnormal retention of renal biomarkers, i.e., blood urea nitrogen and creatinine (Kumar et al. 2013). Also, increase in urea levels might indicate impairment in renal function (Cameron and Greger 1998). So, it is worth to analyze these kidney biomarkers to study the carbon tetrachloride-induced nephrotoxicity.

As expected, administration of CCl_4 (2 ml/kg i.p.) resulted in obvious nephrotoxicity as evident by significant increase in the levels of kidney biomarkers such as blood urea nitrogen (BUN) and creatinine. The observed nephrotoxic effect of CCl_4 was similar to those of previously reported (Ozturk et al. 2003; Ogeturk et al. 2005; Akram and Tembhre 2016). In renal diseases, the serum urea accumulates because its rate of production exceeds the rate of clearance (Mayne 1994). Also, the concentration of creatinine is known to correlate inversely with the degree of glomerular filtration. Hence, creatinine is considered to be

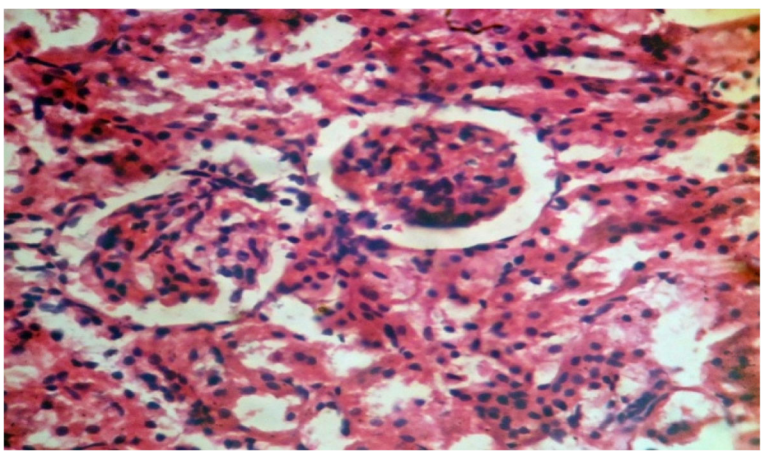

Fig. 1 (Group I)—control group of rats showing normal architecturewith well-defined Bowman's capsule (BW) with glomerulus(G), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), urinary space (US), and vascular pole (VP) (40X, hematoxylin-eosin stain)

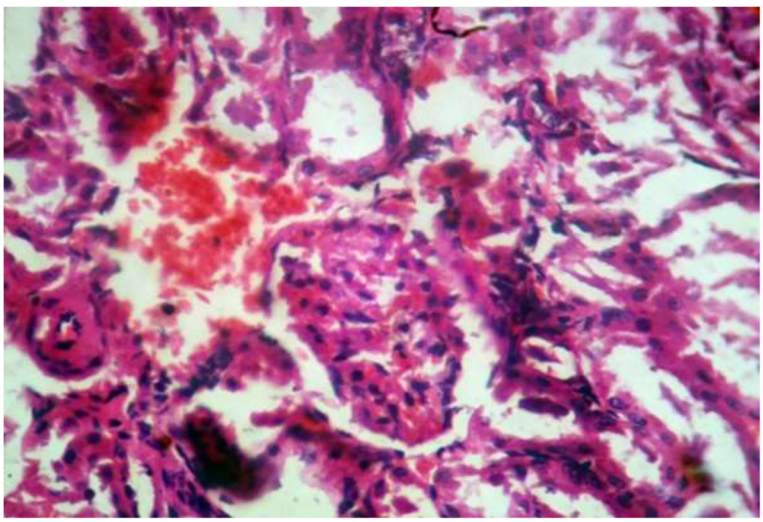

Fig. 2 Shows photomicrographs of kidney of rats inebriated with CCl_4 (2 ml/kg with 50% olive oil, weekly for 30 days)

among the useful markers of the filtration task of kidneys, predominantly that creatinine is excreted only via the kidneys (Pietta 2000). Evaluation of urea and creatinine levels in the serum was taken as an index of nephrotoxicity (Bennit et al. 1982; Anwar et al. 1999; Ali et al. 2001). In our study, the increased level of BUN and creatinine was highly significantly restored near to normal levels when CCl_4-intoxicated rats were given access to EERO at 100 mg/kg and 250 mg/kg of b.wt. in a dose-dependent manner. In agreement with the results of the present study, various investigators reported that the increased levels of BUN and creatinine as a result of toxicities were restored when the rats were treated with herbal extracts (Kannappan et al. 2010; Kore et al. 2011; Hiremath et al. 2012; Akram and Tembhre 2016).

Exposure to CCl_4 induces acute and chronic renal injuries as well as oxidative stress (Churchill et al. 1983; Perez et al. 1987; Ogeturk et al. 2005) and is also known to produce renal diseases in humans (Gosselin et al. 1984; Ruprah et al. 1985). We also studied various biochemicals in kidney homogenate which are involved in oxidative stress. Results obtained shows highly significant decrease ($P < 0.001$) in antioxidant markers, GSH, CAT, SOD, and GPx; however, a highly significant increase ($P < 0.001$) was noticed in the levels of XOD and LPO in the CCl_4-treated group when compared with the control rats. Depletion of endogenous enzymatic and nonenzymatic antioxidants in

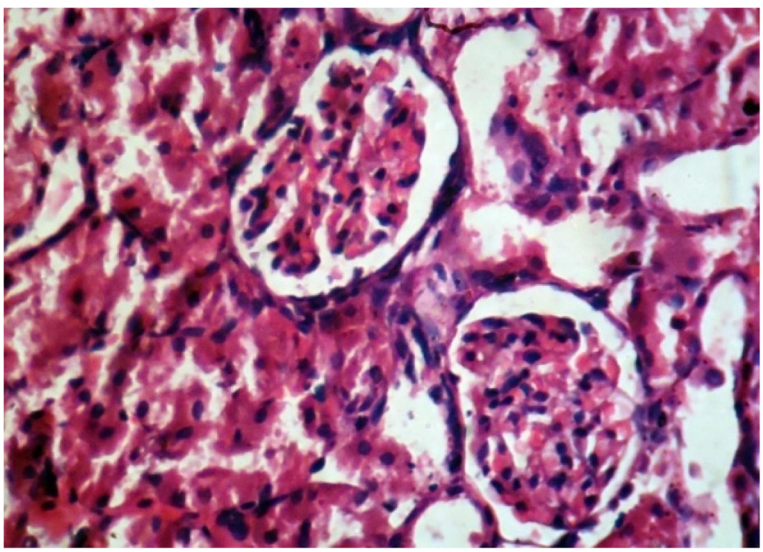

Fig. 3 Shows the kidney section of only EERO treated rats at the dose of 100 mg/kg of body weight

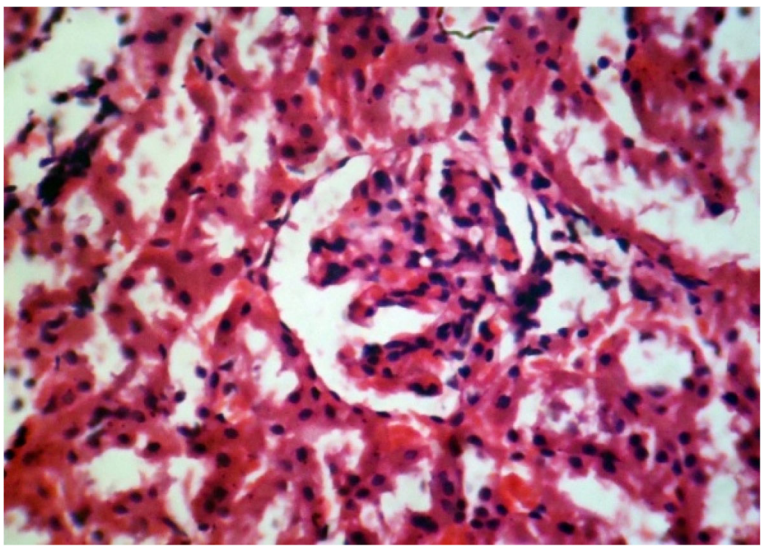

Fig. 4 Shows the kidney section of only EERO-treated rats at the dose of 250 mg/kg of body weight

CCl_4intoxicated group could be attributed to CCl_4 generated cellular ROS production and the subsequent depletion of the antioxidant cellular system (Li et al. 2005; Gowri et al. 2008; Muhammad et al. 2009; Boshy et al. 2017 and Bellassoued et al. 2018). On the other hand, treatment with EERO alone at 100 mg/kg and 250 mg/kg does not show any significant change in the level of these biomarkers as compared with the control group. This clearly showed the nontoxic nature of EERO at both selected doses. However, the altered level of these antioxidant markers, GSH, CAT, SOD, GPx, XOD, and lipid peroxidation (MDA), were highly significantly restored ($P < 0.001$) in dose-dependent manner when the rats were given access to EERO 100 mg/kg and 250 mg/kg of body weight when compared to CCl_4 intoxicated group. However catalase in the case of EERO 100 mg/kg + CCl_4 was restored significantly ($P < 0.05$) and the case of EERO 250 mg/kg + CCl_4 CAT was also restored highly significantly ($P < 0.001$). Our findings were in concordance with other researchers (Venkatanarayana et al. 2012; Karthikeyan et al. 2012; Noorah et al. 2014; Ali and Abdelaziz 2014).

The kidneys of the control and only herb-treated groups showed normal histological features (Figs. 1, 3, and 4) respectively. In group II, i.e., animals intoxicated with CCl_4, there were apparent evidence of renal toxicity as glomerular showed hypertrophy, epithelial layer of Bowman's capsule was degenerated with prominent loss of urinary space between glomerulus and Bowman's

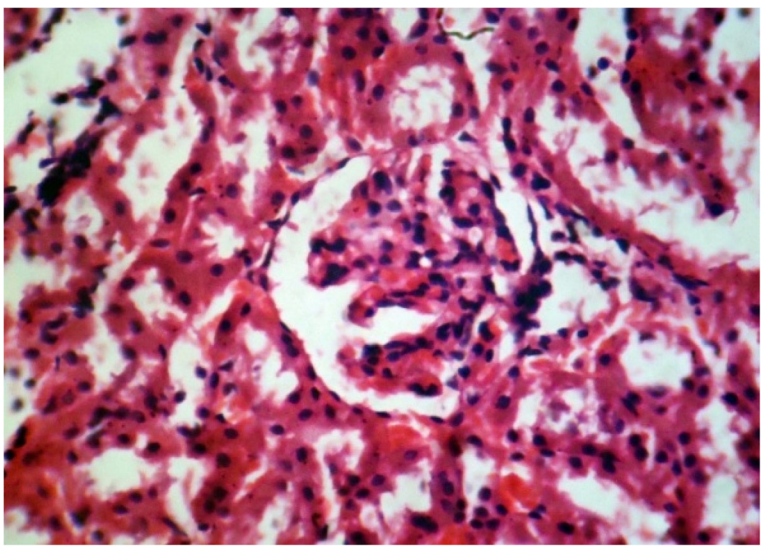

Fig. 5 Shows the photomicrographs of kidney of rats treated with daily dose of EERO 100 mg/kg and CCl_4 once a week

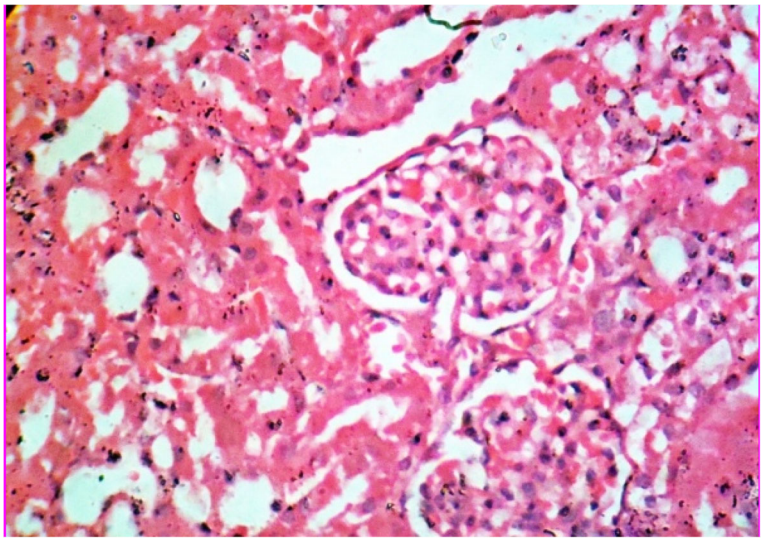

Fig. 6 Shows the photomicrographs of kidney of rats treated with daily dose of 250 mg/kg of EERO and CCl_4 once a week

capsule, inflammatory cell infiltrations, cast formation in renal tubules, disappearance of tubular epithelium, moderate to severe necrosis, congestion, and dilation of blood vessels (Fig. 2). In CCl_4 + 100 mg/kg of EERO-treated group, the glomeruli showed slight hypercellularity (Fig. 5). However, in the case of CCl_4 + 250 mg/kg of body weight group, kidney section revealed normal structure as that of the control group (Fig. 6). Our finding with respect to histopathology was in full agreement with El-kholy et al. 2013 and Foaud et al. 2018.

Conclusion

This study substantiated the scientific evidence in favor of pharmacological uses of *Rosmarinus officinalis*. The findings of our present investigation adequately proved the nephroprotective and antioxidant potentials of ethanolic extracts of *Rosmarinus officinalis* in rats challenged with CCl_4, by preventing the allteration in kidney markers (BUN and creatinine), kidney biochemicals (SOD, CAT, GPx, GSH, and XOD), and also prevents lipid peroxidation. Furthermore, our findings with kidney biomarkers were fully supported by histopathological studies. This protective potential of EERO may be due to its high antioxidant potential.

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Availability of data and materials

The necessary data was obtained during the study. However, on the proposal or request, additional information will be provided by the corresponding author.

Authors' contributions

The work was conceived and premeditated by MAA, MT, RJ, and SK. The experiment was conducted by MAA. The data so obtained was analyzed by MAA, MAS, AJ, UF, and MA. The compilation of the first draft, as well as editing, was done by MAA, MT, and SK. The final manuscript was read and approved by all authors.

Ethics approval

Animal experiments were performed with prior permission from Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Approval No. PBRI/13/IAEC/PN-296a).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

Abraham P, Wilfred G, Cathrine SP (1999) Oxidative damage to the lipids and proteins of the lungs, testis and kidney of rats during carbon tetrachloride intoxication. Clin Chim Acta 289(2):177–179

Akram MA, Tembhre M (2016) *Tagetes minuta* herbal extract: a promising prevention strategy for treatment of nephropathy. Asian J Exp Sci 30(1&2):33–37

Akram MA, Tembhre M, Jehan Q, Sheikh MA, Ahirwar P (2016) Phytochemical screening and evaluation of *in-vitro* antioxidant activity, total phenoloic and total flavonoid content estimations of upper shoot ethanolic extract of *Rosmarinus officinalis*. IJIPSR 4(2):132–142

Ali BH, Ben-Ismail TH, Basheer AA (2001) Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity: influence of gonadectomy and hormonal replacement therapy. Ind J Pharmacol 33:369–373
Ali SAE, Abdelaziz DHA (2014) The protective effect of date seeds on nephrotoxicity induced by carbon tetrachloride in rats. Int J Pharm Sci Rev Res 26(2):62–68
Al-Sereiti MR, Abu-Amer KM, Sen P (1999) Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J Exp Biol 37(2):124–130
Amar Y, Meddah B, Bonacorsi I, Costa G, Pezzino G, Saija A, Cristani M, Boussahel S, Ferlazzo G, Meddah AT (2017) Phytochemicals, antioxidant and antiproliferative properties of *Rosmarinus officinalis* L on U937 and CaCo-2 cells. Iran J Pharm Res 16:315–327
Anwar S, Khan NA, Amin KMY, Ahmad G (1999) Effects of Banadiq-al-buzoor in some renal disorders. Hamdard Medicus, vol. XLII, vol 4. Hamdard Foundation, Karachi, pp 31–36
Azab AE, Albasha MO, Elsayed ASI (2017) Prevention of nephropathy by some natural sources of antioxidants. Yangtze Med 1(4):210–217
Bedevian AK (1994) Illustrated polyglottic dictionary of plant names. Medbouly Library, Cairo
Bellassoued K, Hsouna AB, Athmouni K, Pelt JV, Ayadi FM, Rebai T, Elfeki A (2018) Protective effects of Mentha piperita L. leaf essential oil against CCl_4 induced hepatic oxidative damage and renal failure in rats. Lipids Health Dis 17(9):1–14
Bennit WM, Parker RA, Elliot WC, Gilbert D, Houghton D (1982) Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity. J Infect Dis 145:370–374
Bergmeyer HU, Gawehn K, Grassl M (1974) In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol I, 2nd edn. Academic Press Inc, New York, pp 521–522
Boshy ME, Abdelhamidb F, Richab E, Ashshia A, Gaitha M, Qustya N (2017) Attenuation of CCl_4 induced oxidative stress, immunosuppressive, Hepatorenal damage by Fucoidan in rats. J Clin Toxicol 7:348
Bowers LD (1980) Kinetic serum creatinine assays I. The role of various factors in determining specificity. Clin Chem 26(5):551–554
Cameron JS, Greger R (1998) Renal function and testing of function. In: Davison AM, Cameron JS, Grunfeld JP, DNS K, Rits E, Winearl GC (eds) Oxford textbook of clinical nephrology. Oxford University Press, Oxford, pp 36–39
Churchill DN, Finn A, Gault M (1983) Association between hydrocarbon exposure and glomerulonephritis. An araisal of the evidence. Nephron 33:169–172
Corsonello A, Pedone C, Corica F (2005) Concealed renal insufficiency and adverse drug reactions in elderly hospitalized patients. Arch Intern Med 165: 790–779
Dirks J, Remuzzi G, Horton S, Schieppati A, Hasan Rizvi SA (2006) Diseases of the kidney and the urinary system. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, Jha P, Mills A, Musgrove P (eds) Disease control priorities in developing countries. Oxford University Press, New York, pp 695–706
El-kholy TA, Hassanen NHM and Abbas HY (2013) Protection of the mushroom (shiitake *Lentinus-edodes*) against carbon tetrachloride-induced renal injury in rats. Life Sci J 10(1): 1701–1708
Ellman GL (1959) Tissue sulfydryl groups. Arch Biochem Biophys 82(1):70–71
Farnsworth NR (2005) NAPRALERT database. University of Illinois at Chicago, Chicago [An online database available directly through the University of Illinois at Chicago or through the scientific and technical network [STN] of chemical abstracts services 30.06.200]
Foaud M, Kamel AH, Abd El-Monem DD (2018) The protective effect of N-acetyl cysteine against carbon tetrachloride toxicity in rats. J Basic Appl Zool 79:14
Gosselin RE, Smith RP, Hodge HC (1984) Clinical toxicology of commercial products, 5th edn. Williams and Wilkins, Baltimore
Goth L (1991) A simple method for determination of serum catalase activity and revision of reference range. Clinics Chimica Acta 196:143–152
Gowri S, Manavalan R, Venkappayya D, David R (2008) Hepatoprotective and antioxidant effects of *Commiphora berryi* (Arn) Engl bark extract against CCl_4 induced oxidative damage in rats. Food ChemToxicol 46:3182–3185
Hiremath GS, Padmanabhareddy Y, Hosamath PC (2012) Nephroprotective activity of *Cardiospermum helicacabum* linn. against carbon tetrachloride-induced nephrotoxicity in wistar rats. Ph Tech Med 1(5):187–190
Hoitsma AJ, Wetzels JF, Koene RA (1991) Drug induced nephrotoxicity. Aetiology, clinical features and management. Drug Saf 6(2):131–147
Jaramillo-juarez F, Rodriguez-Vazquez ML, Rincon-Sanchez AR, Consolacion-Martinez M, Ortiz GG, Llamas J (2008) Acute renal failure induced by carbon tetrachloride in rats with hepatic cirrhosis. Ann Hepatol 7(4):331–338
Kannappan N, Madhukar, Mariymmal, Uma SP, Mannavalan R (2010) Evaluation of nephroprotective activity of *Orthosiphon stamineus* Benth extract using rat model. Int J Pharm Tech Res 2(3):209–215
Karthikeyan R, Anantharaman P, Chidambaram N, Balasubramanian T, Somasundaram ST (2012) Padina boergessenii ameliorates carbon tetrachloride induced nephrotoxicity in Wistar rats. J King Saud Univ Sci 2: 227–232
Kokate CK, Purohit AP, Gokhale SB (2001) Text book of pharmacognosy. Nirali Prakashan, Pune, pp 593–597
Kore KJ, Shete RV, Jadhav PJ (2011) Nephroprotective role of *A. marmelos* extract. Int J Res Pharm Chem 1(3):617–623
Kumar Z, Suchetha KN, D'Souza P, Bhargavan D (2013) Evaluation of renal protective activity of *Adhatoda zeylanica* (medic) leaves extract in wistar rats. Nitte Univ J Health Sci 3(4):45–56
Li N, Zhang Q, Song J (2005) Toxicological evaluation of fucoidan extracted from *Laminaria japonica* in Wistar rats. Food ChemToxicol 43:421–426
Maajida AM, Gayathri RV, Priya V (2017) Preliminary phytochemical analysis and estimation of total phenolic content in rosemary extract. Int J Pharm Sci Rev Res 44(2):207–209
Mayne PD (1994) The kidneys and renal calculi. In: Clinical chemistry in diagnosis and treatment, 6th edn. Edward Arnold Publications, London, pp 2–24
Muhammad RK, Wajiha R, Gul NK, Rahmat AK, Saima S (2009) Carbon tetrachloride-induced nephrotoxicity in rats: protective role of *Digera muricata*. J Ethnopharmacol 122:91–99
Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK, Mohan H (2003) Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. Phytochemistry 63:97–104
Noguchi T, Fong KL, Lai EK, Alexander SS, King MM, Olson L, Poyer JL, Mccay PB (1982) Specificity of a phenobarbital-induced cytochrome P450 for metabolism of carbon tetrachloride to the trichromethyl radical. Biochem Pharmacol 31:615–624
Noorah S, Al-Sowayan, Mousa HM (2014) Ameliorative effect of olive leaf extract on carbon tetrachloride-induced nephrotoxicity in rats. Life Sci J 11(5):238–242
Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M (2005) Cafeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. J Ethnopharmacol 97:273–280
Ohkawa H, Ohishi N, Vagi K (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 95(2):351–358
Olagunjua JA, Adeneyeb AA, Fagbohunkac BS, Bisugac NA, Ketikuc AO, Benebod AS (2009) Nephroprotective activities of the aqueous seed extract of *Carica papaya* Linn. in carbon tetrachloride induced-renal injured wistar rats: a dose and time dependent study. Biol Med 1(1):11–19
Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K (2003) Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology 62(2):353–356
Paoletti F, Aldinucci D, Mocali A, Caparrini A (1986) A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal Biochem 156:536–541
Perez AJ, Courel M, Sobrado J, Gonzalez L (1987) Acute renal failure after topical application of carbon tetrachloride. Lancet 1:515–516
Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63(7):1035–1042
Porter GA, Bennett WM (1981) Nephrotoxic acute renal failure due to common drugs. Am J Physiol 241(7):1–8
Raghuramulu N, Nair MK, Kalyanasundaram S (1983) A manual of laboratory technique. NIN. Indian Council of Medical Research, Hyderabad, p 92
Rampart M, Beetens J, Bult H, Herman A, Parnham M, Winkelmann J (1986) Complement-dependent stimulation of prostacyclin biosynthesis: inhibition by rosmarinic acid. Biochem Pharmacol 35:1397–1400
Recknagel RO, Glende EA, Dolak JA, Waller RL (1989) Mechanism of carbon tetrachloride toxicity. PharmacolTher 43:139–154
Rungby J, Ernst E (1992) Experimentally induced lipid peroxidation after exposure to chromium, mercury or silver: interactions with carbon tetrachloride. Pharmacol Toxicol 70(3):205–207
Ruprah H, Mant TGK, Flanagan RJ (1985) Acute carbon tetrachloride poisoning in 19 patients: implications for diagnosis and treatment. Lancet 1:1027–1029
Sanzgiri UY, Srivatsan V, Muralidhara S, Dallas CE, Bruckner JV (1997) Uptake, distribution, and elimination of carbon tetrachloride in rat tissues following inhalation and ingestion exposures. Toxicol Appl Pharmacol 143(1):120–129
Sharma V, Sharma A, Kansal L (2010) The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. Food Chem Toxicol 48:928–936

Slot C (1965) The significance of the systemic arteriovenous difference in creatinine clearance determinations. Scand J Clin Lab Invest 17:201–208
Srinath RK, Bhah B, Verghese C, Ramadoss A (2005) Responding to threat of chronic diseases in India. Lancet 366(9498):1746–1751
Stefanovits-Banyai E, Tulok M, Hegedus A, Renner C, Varga S (2003) Antioxidant effect of various rosemary (*Rosmarinus officinalis* L.) clones. Acta Biol Szeged 47:111–113
Talka H, Schubert GE (1965) Enzymatic urea determination in the blood and serum in the Warburg optical test. Klin Wochschr 19(43):174
Tiffany TO, Jansen J, Burtis CA, Overton JB, Scott CD (1972) Enzymatic kinetic rate and end-point analysis of substrate, by use of a GeMSAEC fast analyze. Clin Chem 18:829
Venkatanarayana G, Sudhakara P, Indra SP (2012) Protective effects of curcumin and vitamin E on carbon tetrachloride-induced nephrotoxicity in rats. EXCLI J 11:641–650
Wendel A (1980) Glutathione peroxidases. In: Jakoby WB (ed) Enzymatic basis of detoxification. Academic Press, New York, pp 333–353
Young DS (1975) Effects of drugs on clinical laboratory tests. Clin Chem 21(5):1D–432D