Pharmaceutical Sciences
December 2016, 22, 227-233
doi: 10.15171/PS.2016.36
http://journals.tbzmed.ac.ir/PHARM

Research Article

Effects of Sodium Selenite on Formaldehyde Induced Renal Toxicity in Mice

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Article Info

Article History:
Received: 3 February 2016
Accepted: 7 August 2016
ePublished: 30 December 2016

Keywords:
-Formaldehyde
-Selenium
-Kidney
-Mouse

ABSTRACT

Background: Formaldehyde is widely used for industrial applications. Renal injury is an adverse effect associated with formaldehyde. Few studies have explored the potential benefits of protective factors on formaldehyde induced renal toxicity. This study evaluated the dose dependent effects of sodium selenite on the biochemical and histopathological effects of formaldehyde on murine kidney.

Methods: Forty eight adult Balb/c male mice were randomized into six groups: a control group, a formaldehyde group and experimental III-VI groups. Formaldehyde group was injected with 10 mg/kg formaldehyde and groups III-VI received intraperitoneally doses of 0.1, 0.2, 0.4, 0.8 mg/kg selenium. After two weeks, a stereological study was done in accordance with the principle of Cavalieri and serum concentrations of urea and creatinine were measured. Data were analyzed using ANOVA and SPSS software.

Results: Glomerosclerosis, necrosis and vacuolization were observed in the convoluted tubules of animals treated with formaldehyde. The biochemical markers, volume and count of glomeruli in the group treated with formaldehyde was significantly difference compared to the control group ($P<0.05$). The volume of the glomeruli in the group treated with 0.2 and 0.4 mg selenium and urea level in the group treated with 0.4 and 0.1 mg/kg selenium was significantly difference compared to the control group ($P<0.05$). The count of glomeruli and creatinine level in the selenium group was significantly difference compared to the control group ($P \leq 0.0001$).

Conclusions: A dose of 0.2 mg/kg of sodium selenite caused partial protective effect on the renal tissue and function in exposed to formaldehyde.

Introduction

Formaldehyde is an important environmental pollutant.[1] It is widely used in the manufacture of wood and MDF, rubber and in textile industries. In addition, it is used in the preparation of herbicides and fungicides for farm use, and in hospitals for pathology and histology in the laboratory as a fixative and also as part of preservative materials for dental laboratories. The limit for occupational exposure, up to 8 h long-term exposure, is 0.75 ppm in air and short-term 15 min exposure is 2 ppm.[2,3] However, there are also low levels of formaldehyde in detergents, disinfectant solutions, cosmetics and toothpaste. Hence, nowadays, each

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person is either directly or indirectly exposed to formaldehyde.[1] Many studies have demonstrated the adverse effects of formaldehyde on the respiratory, cardiovascular and urogenital systems.[4-7] It has been shown that formaldehyde leads to degeneration of the glomeruli, dilatation and vacuolization of renal tubules in rat models.[8] After exposure to formaldehyde, an increase in serum urea, creatine and malondialdehyde levels is observed while the levels of glutathione peroxidase and superoxide diminished. Increased levels of malondialdehyde (MDA) and low level of antioxidants disturb the balance of oxidant and antioxidant, thereby leading to oxidative stress.[9,10] Formaldehyde increases free radicals that must remain inactive for normal cell function. Free radicals are neutralized by the actions of a variety of antioxidants. These antioxidants are divided into enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic antioxidants including vitamins C and E, pyruvate, carnitine and selenium.[11]

Selenium is a trace element found in large quantities in dairy products, liver and cereals and plays a vital role in the body's metabolic system. Selenium is a component of glutathione peroxidase and is involved in the activation of antibody biosynthesis and coenzyme Q (a component of the mitochondrial electron transport system), regulation of the flow of ions through the cell membrane and neutralizes the excessive amount of heavy metals.[12,13] It has been reported that selenium is effective in the treatment of poisoning with cadmium and lead compounds.[14,15] There are few reports on the protective effects of antioxidants on kidney tissue. The aim of this study was to evaluate the effect of different doses of sodium selenite on the biochemical and histopathological parameters in the kidney of mice treated with formaldehyde.

Material and Methods

In this experimental study, after approval by the Ethics Committee, 48 adult Balb/c male mice were purchased from the Razi Institute. All experiments were performed in compliance with the laws for animal trial. The mice were randomized to six groups. The control group received no injection. The second group was injected with 10 mg/kg formaldehyde.[16] The third to sixth groups received intraperitoneally 0.1- 0.2- 0.4- 0.8 mg/kg selenium plus 10 mg/kg formaldehyde for 14 days. Then, the mice were anesthetized with chloroform and the left kidney was placed into fixative solution.

Stereological evaluation of kidney

The total volume of each kidney and glomeruli was calculated using the principle of Cavalier. For this purpose, after tissue processing, 50 sections from each sample and 5 fields from each section were randomly selected. Images were photographed using a microscope is equipped with a digital camera and magnification ×40. Finally, the number of points hitting the kidney transects were counted Following formula was used to calculate the kidney volume:

$$V = \frac{\sum pi \times a(p) \times t}{M^2} \quad \text{Eq.(1)}$$

Σpi: Total points of grid hitting the kideny, a/ (p): the area around each point of grid, t: the thickness of each slice and M^2: magnification. Glomerular volume was calculated from the following formula:

$$V_{total}\left(Glomeruli\right) = V_V\left(Glomeruli\right) \times V\left(refrence\right) \quad \text{Eq.(2)}$$

In the formula for calculating the Vv, below formula was used.[17,18]

$$Vv = \frac{\sum p\left(\text{Glomeruli}\right)}{\sum p\left(\text{Cortex}\right)} \quad \text{Eq.(3)}$$

To determine the number of glomeruli, after randomly placing the grid on the images, glumeruli in the reference section were counted. Finally, the final number of glomeruli was calculated by the following formula. ΣQ: Total points of grid hitting the glomeruli, a/ (p): the area around each point of grid, h: dissector height and ΣP: the number of frames.

$$N = \frac{\sum Q}{a\left(p\right).h.\sum P} \times V_{Cortex} \quad \text{Eq.(4)}$$

Urea and creatinine serum level

To evaluate the serum urea and creatinie level, 1 ml of blood were collected and centrifuged at 1000 rpm. Urea and creatinine serum level were measured using pars azmoon kits and an Auto analyzer (B 1000, Biotecnica instruments).

Statistical analysis

Data are presented as Mean±SD. The normality of data was assessed using the Kolmogorov-Smirnov test. Data were analyzed using ANOVA and Tukey test. P value less than 0.05 was considered as significant.

Histological findings in experimental groups

Images of the kidneys were evaluated by two separate individual. For the control samples, glomeruli and tubules were normal. The interstitial tissue and renal arteries were also normal. Collapse, glomerulosclerosis, necrosis, vacuolization and cast were observed after administration of formaldehyde. In addition, interstitial and renal arteries were normal. Collapse of glomeruli was observed in mice treated with 0.1 mg selenium. In addition, vacuolization, necrosis

and cast were observed in convoluted tubules. Interstitial tissue and renal arteries were normal. Glomeruli, convoluted tubules and renal arteries were normal in group treated with 0.2 mg/kg selenium. Glomerular lobulation as well as mild focal inflammation were observed in group treated with 0.4 mg/kg selenium. Vacuoles, cast and necrosis were observed in convoluted tubules. Glomeruli were normal in group treated with 0.8 mg/kg selenium. Vacuolization, necrosis and cast were observed in convoluted tubules. Interstitial tissue and renal arteries were normal (Figure 1and 2).

Kidney volume, glomerular volume and glomerular count in experimental groups

As shown in Figure 3-5, kidney volume in group treated with formaldehyde was significantly difference compared to the control group ($P \leq 0.0001$). In addition, the kidney volume was significantly difference in groups treated with 0.1 and 0.4 mg/kg selenium compared to this value of the formaldehyde group ($P \leq 0.0001$). Glomeruli count in group treated with formaldehyde was significantly difference compared to the control group ($P \leq 0.0001$). In addition, glomerular volume in group treated with 0.1, 0.2, 0.4 and 0.8 mg/kg selenium was significantly difference compared to the formaldehyde group ($P \leq 0.001$).

The statistical analysis showed a significant difference between values of count of glomeruli in the formaldehyde group compared to the control group ($P < 0.01$). In addition, the count of glomeruli in group treated with 0.4 mg/kg selenium was significantly difference compared to the formaldehyde group ($P \leq 0.0001$).

Level of urea and creatinine level in experimental groups

Data of serum urea and creatinine are presented in Table 1. Urea level in the formaldehyde group was significantly difference compared to the control group ($P \leq 0.0001$). There was a significant difference between value of urea in group treated with 0.8 mg selenium compared to the formaldehyde group ($P < 0.05$). Urea level was not significantly different in the group treated with formaldehyde compared to groups treated with 0.1, 0.2 and 0.4 mg/kg selenium ($P > 0.05$).

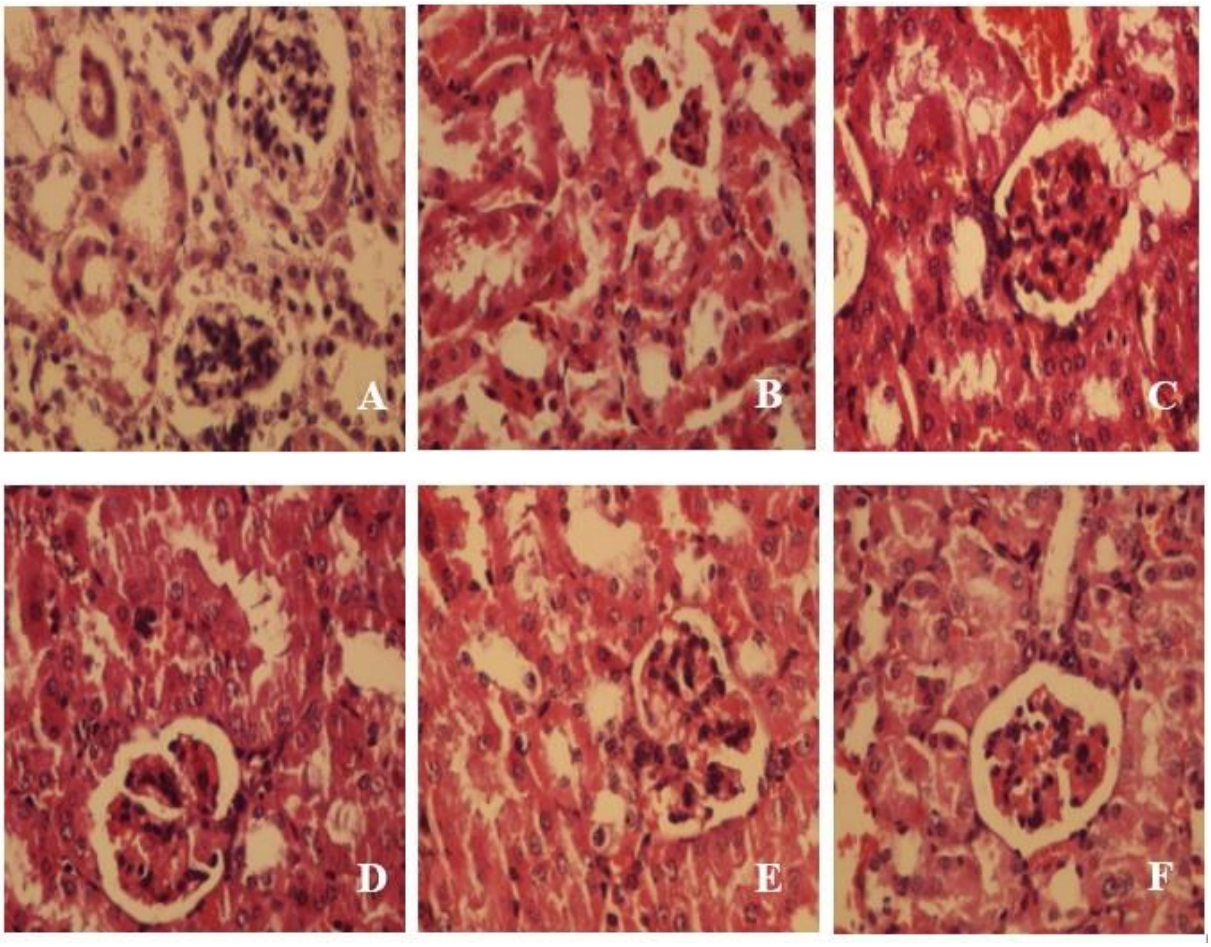

Figure 1. (A-F) are images of renal glomeruli in the control group, formaldehyde group, 0.1 mg/kg selenium group, 0.2 mg/kg selenium group, 0.4 mg/kg selenium group and 0.8 mg/kg selenium group . Magnification × 40, H&E staining.

Level of creatinine in the formaldehyde group was significantly difference compared to the control group (P ≤ 0.0001). Level of creatinine in the formaldehyde group was not statistically significance compared to the 0.1, 0.2, 0.4 and 0.8 mg selenium (P = 1.000). The level of creatinine in the selenium group was statistically significant compared to the control group (P ≤ 0.0001).

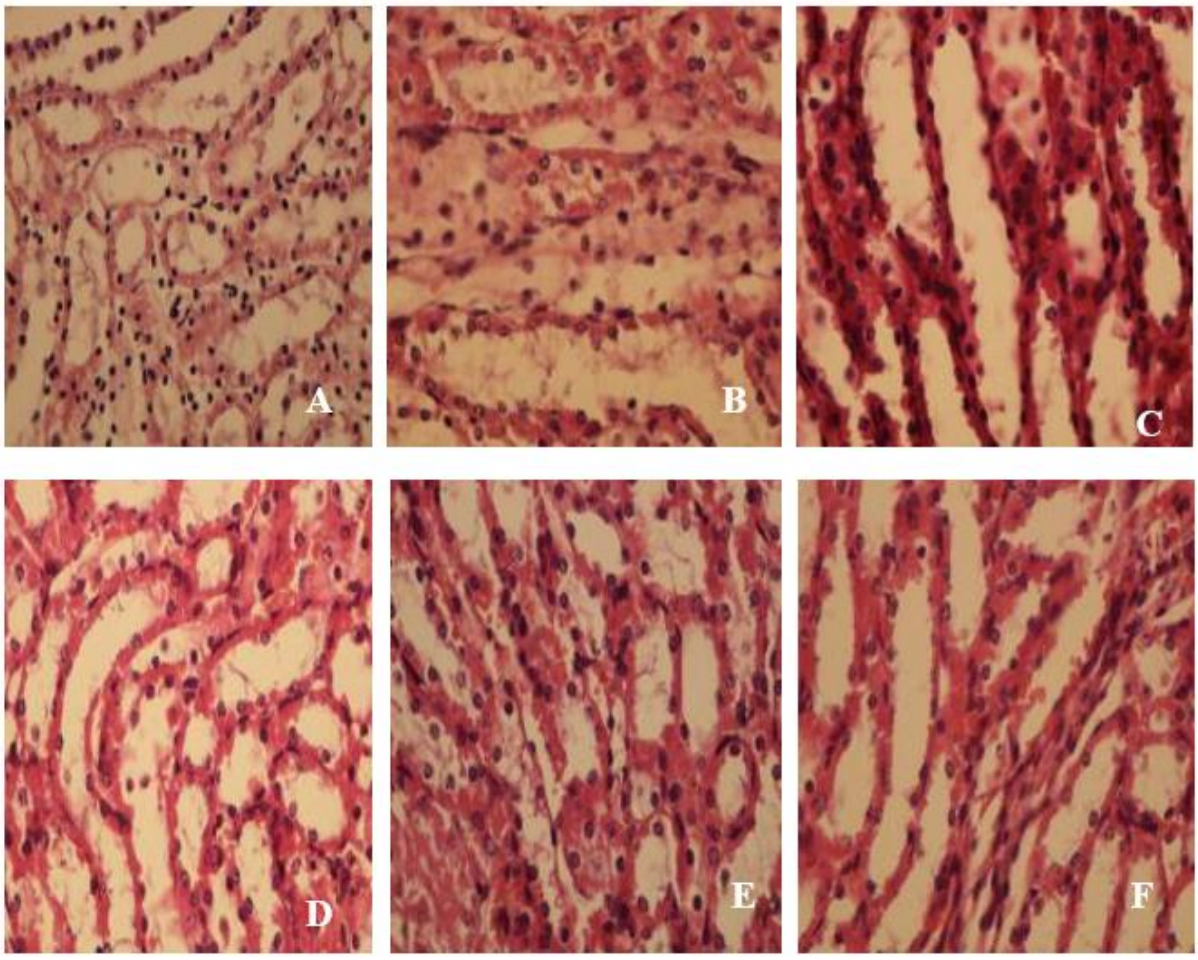

Figure 2. (A-F) are images of convoluted tubules in the control group, formaldehyde group, 0.1 mg/kg selenium group, 0.2 mg/kg selenium group, 0.4 mg/kg selenium group and 0.8 mg/kg selenium group . Magnification × 40, H&E staining.

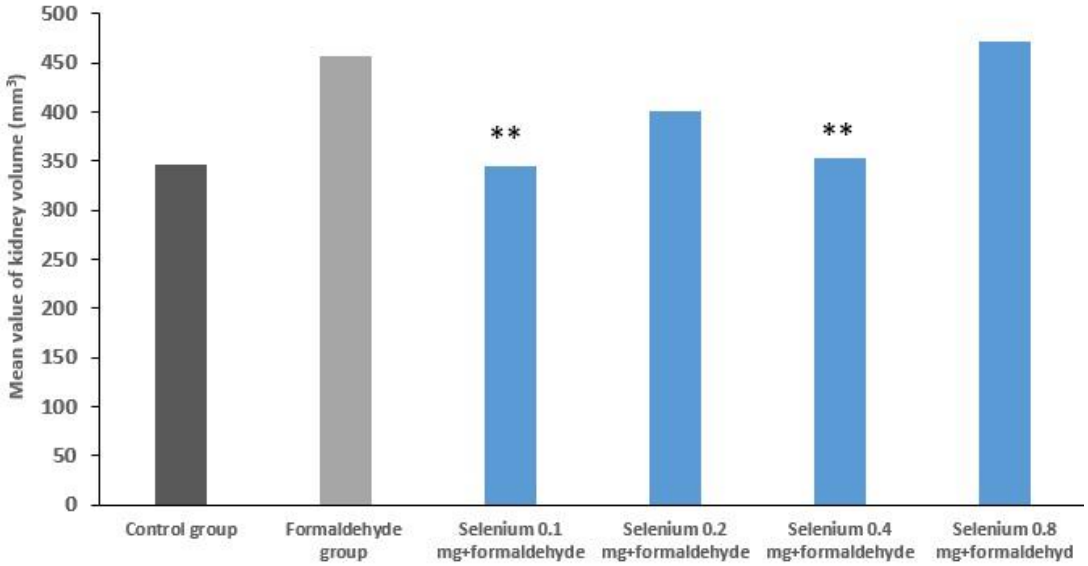

Figure 3. Kidney volume in experimental groups. **P≤ 0.0001 compared to the formaldehyde group.

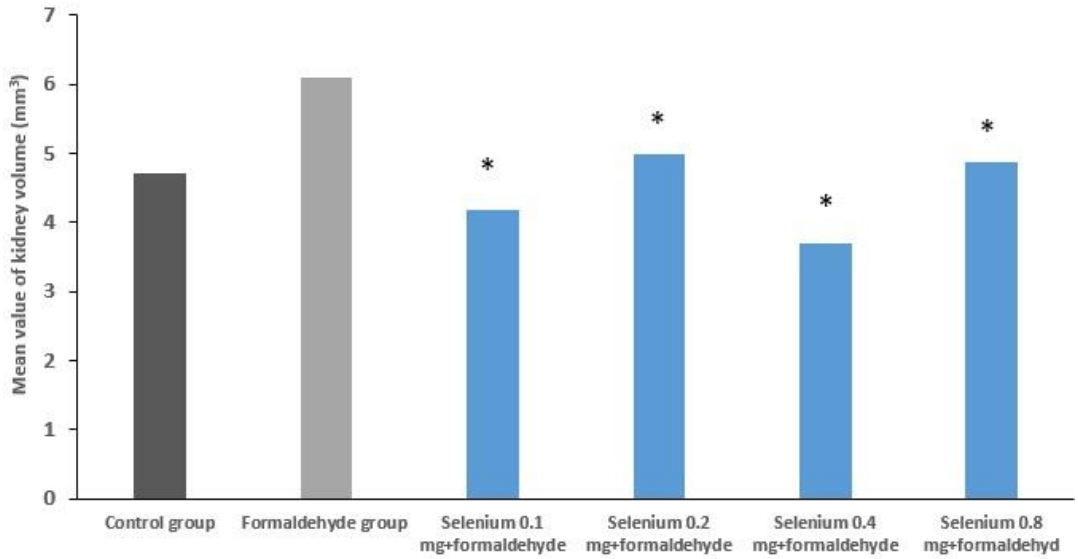

Figure 4. Glomerular volume in experimental groups. *P≤0.001 compared to the formaldehyde group.

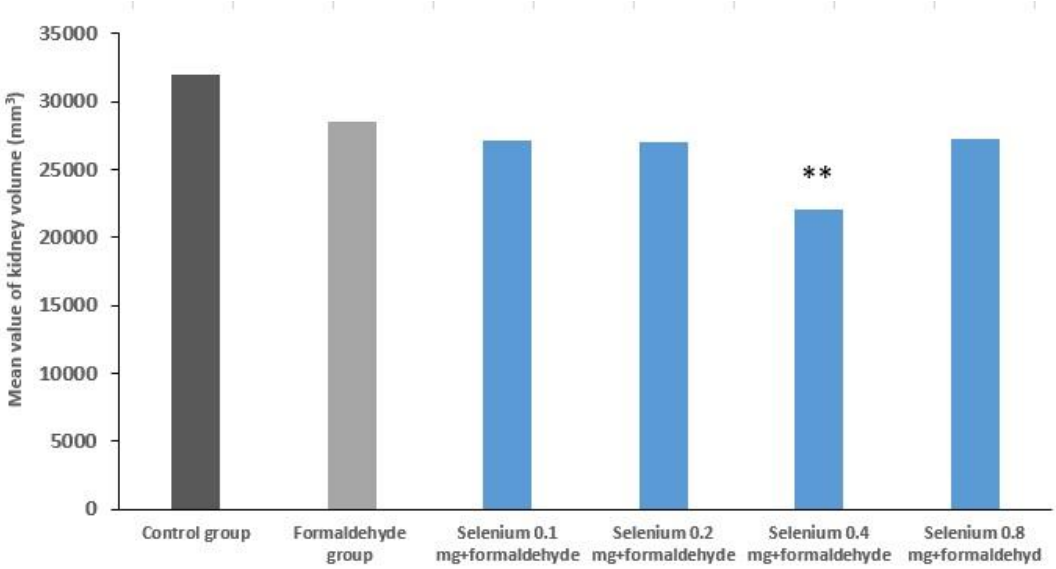

Figure 5. Glomerular count in experimental groups. **P≤ 0.0001 compared to the formaldehyde group.

Table 1. Level of urea and creatinine in experimental groups.

Experimental groups	Creatinine level	P value	Urea level	P value
Control	0.48±0.06	-	29.06±3.50	-
Formaldehyde	0.71±0.06	0.001	54.25±3.53	0.001
Selenium 0.1 mg	0.72±0.04	0.001	44.46±8.77	0.027
Selenium 0.2 mg	0.71±0.04	0.001	41.68±8.9	0.108
Selenium 0.4 mg	0.72±0.08	0.001	61.38±8.44	0.001
Selenium 0.8 mg	0.68±0.06	0.001	39.57±3.36	0.257

P values expressed compared to the control group.

Discussion

Overall, the results showed that a dose of 0.1 mg of formaldehyde causes adverse effects on kidney tubules, as well as the glomeruli. The effects of selenium supplementation for renal volume were dose-dependent. In addition, formaldehyde had negative effects on urea and creatinine levels. Kidney function is determined by measurement of urea and creatinine. In their study, Boj et al. showed that the serum level of urea and creatinine, after 48 h of exposure to formaldehyde significantly increased at both low concentrations and high doses of formaldehyde.[10] Similarly, in the present study after administration of 10 mg of formaldehyde, the levels of urea and creatinine increased as compared to the control group.

Studies showed that exposure to formaldehyde at a rate of 1 to 1.9 ppm for 18 weeks causes degenerative changes in the renal tubules, vacuolization and focal congestion, as well as renal glomeruli and vascular congestion.[19] In the results of another study, degeneration of the glomeruli, thickening of the kidney tubule basement membrane, dilatation, necrosis and vacuole of the distal tubules of rats after exposure to formaldehyde were observed. The level of glutathione peroxidase and superoxide dismutase reduced while levels of malondialdehyde (MDA), a marker of oxidative stress increased. Treatment with melatonin enhanced the level of antioxidant enzymes and reduced the level of malondialdehyde. In addition, kidney damage significantly reduced and the injury improved except a mild dilatation of the renal tubules.[8]

Studies demonstrated that the administration of 0.1 mg / kg selenium results in the neutralization of oxidants and elimination of cadmium toxicity in the kidneys of rats. In addition, it causes a reduction in the level of malondialdehyde, inflammatory markers such as IL-6, IL-10, tumor necrosis factor-alpha and interleukin-ß. Also, it increases the level of enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase and catalase.[15] Formaldehyde increases protein-DNA cross-links in culture. In addition, it increased the levels of malondialdehyde, NF-KB necrosis factor and AP-1, but reduced the level of superoxide dismutase and glutathione peroxidase.

Selenium pretreatment attenuates formaldehyde-induced genotoxicity in A549 cell lines. The addition of selenium to cell culture before exposure to formaldehyde, reduced protein-DNA cross-links, level of malondialdehyde, NF-KB and AP-1 protein but increased superoxide dismutase and the level of glutathione peroxidase.[19] In this study, with respect to the rapid absorption of formaldehyde in the blood, the likely toxic effects of formic acid is due to formic acid as its main metabolite. Formic acid is excreted from the body through the liver or urine. Increasing the concentration of formic acid inhibits the respiratory cycle while cell anaerobic respiration leads to the production of lactic acid. Lactic acid causes reduction in the secretion of formic acid into the renal tubules and as a result leads to cell toxicity.[19,20]

This suggests that antioxidants are like a double-edged sword, and that the appropriate dose and duration of their use have good effects while inappropriate dose or time reverses the effects.[21,22] Although, the exact mechanism of selenium nephrotoxicity is not understood, it is postulated that selenium acts as a scavenger of reactive oxygen species in the kidney tissue. Finally, it is recommended for future studies in biochemical analysis such as measurement of antioxidant capacity, markers of inflammation and oxidative stress. In addition, the effects of selenium supplementation is investigated in the different time of treatment, on kidneys exposed to formaldehyde.

Conclusion

Histopathological results showed that formaldehyde causes adverse effects on kidney tissue in mice. In addition, an increase in the kidney weight and the urea and creatinine concentration was observed in the formaldehyde group. Administration 0.2 mg/kg sodium selenite caused protective effect on renal tissue exposed to formaldehyde during 14 days.

Acknowledgment

This study is derived from a research project that confirmed by Students Research Committee of Gonabad University of Medical Sciences. So, the authors wish to thank them for their assistance.

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Inci M, Zararsız I, Davarcı M, Görür S. Toxic effects of formaldehyde on the urinary system. Turk J Urol . 2013;39(1):48-52. doi:10.5152/tud.2013.010
2. Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, El Ghissassi F. Meeting report: summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2- propanol. Environ Health Perspect. 2005;113(9):1205-8. doi:10.1289/ehp.7542
3. Naya M, Nakanishi J. Risk assessment of formaldehyde for the general population in Japan. Regul Toxicol Pharmacol. 2005;43(3):232-48. doi:10.1016/j.yrtph.2005.08.002
4. Mohammadi S, Safari F, Seyedi Z, SeyedHosseini E, Karimi F, Mohammadi M, et al. Effect of different doses of N-acetyl cysteine on biochemical and histopathological parameters in kidney of formalin-treated mice. J Kerman Univ Med Sci . 2015;23(5):607-17.
5. Arican RY, Sahin Z, Ustunel I, Sarikcioglu L,Ozdem S, Oguz N. Effects of formaldehyde inhalation on the junctional proteins of nasal respiratory mucosa of rats. Exp Toxicol Pathol. 2009;61(4):297-305. doi:10.1016/j.etp.2008.09.005
6. Gulec M, Gurel A, Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol Cell Biochem. 2006;290(1-2):61-7. doi:10.1007/s11010-006-9165-z

7. Mohammadi S. Protective effect of N-acetyl cysteine against formaldehyde-induced neuronal damage in cerebellum of mice**.** Pharm Sci. 2014;20(2):61-5
8. Zararsiz I, Sarsilmaz M, Tas U, Kus I, Meydan S, Ozan E. Protective effect of melatonin against formaldehyde-induced kidney damage in rats. Toxicol Ind Health. 2007;23(10):573–9. doi:10.1016/0306-4522(96)00155-8
9. Zararsiz I, Sonmez MF, Yilmaz HR, Tas U, Kus I, Kavakli A, et al. Effects of ω-3 Essential fatty acids against formaldehyde-induced nephropathy in rats. Toxicol Ind Health. 2006;22(5):223–9. doi:10.1191/0748233706th260oa
10. Boj JR, Marco I, Cortés O, Canalda C. The acute nephrotoxicity of systemically administered formaldehyde in rats. Eur J Paediatr Dent. 2003;4(1):16–20.
11. Arthur JR. New metabolic roles for selenium. Proc Nutr Soc. 1994;53(3):615-24.
12. Mousavi M, Heidari E, Rayman MP, Tara F, Boskabadi H, Mohammadi S, et al. Effects of selenium supplementation on soluble fms-like tyrosine kinase-1 and glutathione peroxidase levels and the plasminogen activator inhibitor-1: Plasminogen activator inhibitor-2 ratio in pregnant women. Shiraz E Med J. 2015;16(3):e2217. doi:10.17795/semj21596
13. Mohammadi S, Movahedin M, Mowla SJ. Up-regulation of CatSper genes family by selenium. Reprod Biol Endocrinol. 2009;7(1):126. doi:10.1186/1477-7827-7-126
14. Othman AI, Missiry MAE. Role of selenium against Lead Toxicity in Male Rats. J Biochem Mol Toxicol. 1998;12(6):345-9. doi:10.1002/(sici)1099-0461(1998)12:6<345::aid-jbt4>3.0.co;2-v
15. El-Boshy ME, Rishab EF, Abdelhamidb FM, Mubarakc MS, Haddad TB. Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. J Trace Elem Med Biol. 2015;29:104-10. doi:10.1016/j.jtemb.2014.05.009
16. Tajaddini Sh, Ebrahimi S, Shirinbayan P, Bakhtiyari M, Behnam B, Joghataei MT, et al. Protective Effects of Manganese on the Testis Structure and Sperm Parameters of Formalin Treated Mice. J Isfahan Med Sci. 2013;31(243):1018-32.
17. Jalali M, Nikravesh MR, Moeen AA, Karimfar MH, Mohammadi Sh , Saidi nejat Sh, et al. Inductive Role of Type IV Collagen in nephrogenesis. Urol J. 2009;4(6):289-94.
18. Mohammadi S, Pakrouh Z, Teimouri M, Hagi pour S, Karim Mi, Mohammadi M, et al. Effects of drug substance crystal (methamphetamine) on histopathology and biochemical parameters of kidney in adult male mice. J Kurdistan Univ Med Sci. 2015;20(5):83-90.
19. Davarian A, Azarhoush R, Fazeli AH, Hashemnezhad H, Golalipour MJ. Histopathologic effects of formaldehyde exposure on rat kidney. J Mazandaran Univ Med Sci. 2007;16(56):101-7.
20. Shi YQ, Chen X, Dai J, Jiang ZF, Li N, Zhang BY, et al. Selenium pretreatment attenuates formaldehyde-induced genotoxicity in A549 cell lines. Toxicol Ind Health. 2014;30(10):901-9. doi:10.1177/0748233712466129
21. Mohammadi S, Zamani E, Mohadeth Z, Mogtahedi F, Chopan H, Moghimi F, et al. Effects of different doses of simvastatin on lead-induced kidney damage in Balb/c male mice. Pharm Sci. 2015; 20(4):157-62.
22. Mousavi M, Heidari E, Rayman MP, Tara F, Boskabadi H, Mohammadi S, et al. Effects of selenium supplementation on soluble fms-like tyrosine kinase-1 and glutathione peroxidase levels and the plasminogen activator inhibitor-1: Plasminogen activator inhibitor-2 ratio in pregnant women. Shiraz E Med J. 2015;16(3):e21596. doi:10.17795/semj21596