FABAD J. Pharm. Sci., 45, 1, 37-44, 2020

RESEARCH ARTICLE

Antioxidant Effect of Sildenafil on Cadmium-Induced Liver, Lung and Kidney Injury

Ahmet Hüsamettin BARAN[*°], Ahmet BERK[**], M. Bahadır KAYMAZ[***], Göknur AKTAY[****]

Antioxidant Effect of Sildenafil on Cadmium-Induced Liver, Lung and Kidney Injury

SUMMARY

The aim of this study is to investigate the protective effect of sildenafil against cadmium-induced liver, lung and kidney toxicity. In total of 28 Sprague Dawley female rats included in this study were divided into four groups: control, Sil, Cd, Sil+Cd groups. CdCl2 and Sil citrate were dissolved in distilled water, the injection volume adjusted to 0,5 ml / rat. On the 7th day, a single dose of 3,7 mg/kg Cd was injected ip in the morning and Sil was administered in drinking water for 10 days. On the 10th day of the experiment, 72 hours after Cd administration, the rats were sacrificed under anesthesia and the lungs, kidneys and livers of the rats were isolated and Thiobarbituric acid reactive substances (TBARs), GSH, total thiol (T-SH) levels in these tissues were evaluated. Tissue damage was assessed by serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN) and creatine kinase (CK-MB). It was found that cadmium significantly increased AST, ALT, creatinine, BUN, CK-MB and TBARs levels compared to the control group, and significantly decreased GSH and T-SH levels. In sildenafil + cadmium administration, TBARs levels significantly decreased while GSH and T-SH levels significantly increased compared to cadmium group. The protective effect of sildenafil against multiple organ damage caused by cadmium may be due to its antioxidant properties.

Key Words: *Antioxidant system, inflammation, cadmium, oxidative stress, sildenafil, heavy metal toxicity.*

Kadmiyumun Neden Olduğu Karaciğer, Akciğer ve Böbrek Hasarı Üzerine Sildenafil'in Antioksidan Etkisi

ÖZ

Bu çalışmanın amacı kadmiyum ile indüklenen karaciğer, akciğer ve böbrek toksisitesine karşı sildenafilin koruyucu etkisini incelemektir. Bu çalışmaya dahil edilen toplam 28 adet Sprague Dawley cinsi dişi sıçan dört gruba ayrıldı: kontrol, Sil, Cd, Sil+Cd grupları. CdCl2 ve Sil distile su içinde çözüldü, enjeksiyon hacmi 0,5 ml/sıçan olacak şekilde ayarlandı. 7. gün sabah tek doz 3,7 mg/kg Cd ip enjeksiyon halinde, Sil ise 10 gün boyunca içme suyu içinde uygulandı ve 10. gün, Cd uygulamasından 72 saat sonra sıçanlar anestezi altında kesilerek serum, akciğer, böbrek ve karaciğer alınıp dokularda TBARS, GSH, T-SH seviyeleri değerlendirildi. Doku hasarları serum ALT, AST, kreatinin, BUN ve CK-MB ile değerlendirildi. Kadmiyum'un AST, ALT, kreatinin, BUN ve CK-MB ve TBARs düzeylerini kontrol grubuna göre istatistiksel açıdan anlamlı bir şekilde artırdığı, GSH ve T-SH düzeylerini ise anlamlı derecede azalttığı tespit edilmiştir. Sildenafil+kadmiyum uygulamasında ise, kadmiyum grubuna göre TBARs düzeyi anlamlı derecede azalırken, GSH ve T-SH düzeyleri anlamlı derecede artmıştır. Sildenafil'in, kadmiyum'un neden olduğu çoklu organ hasarına karşı koruyucu etkisinin antioksidan özelliğinden kaynaklandığı söylenebilir.

Anahtar Kelimeler: *Antioksidan sistem, enflamasyon, kadmiyum, oksidatif stres, sildenafil, ağır metal toksisitesi*

Received: 22.01.2020
Revised: 16.02.2020
Accepted: 21.02.2020

[*] ORCID: 0000-0003-0830-313X Department of Pharmacy Services, Sinop University Durağan Vocational School, 57200 Sinop
[**] ORCID: 0000-0002-0828-6520 Department of Pharmacy, Elazığ Fethi Sekin City Hospital, 44280 Elazığ
[***] ORCID: 0000-0002-0033-898X Department of Pharmacology, Inönü University Faculty of Pharmacy, 44280 Malatya
[****] ORCID: 0000-0002-1646-8674 Department of Pharmacology, Inönü University Faculty of Pharmacy, 44280 Malatya

[°] Corresponding Author; Ahmet Hüsamettin Baran
Tel: 05534514950
Email: abaran@sinop.edu.tr

INTRODUCTION

Heavy metal toxicity is a public health problem that can be caused by both environmental and occupational resources, threatens the environment and public health due to the widespread presence of metals in the earth's crust, and which has serious consequences such as serious organ damage and even death in acute or chronic exposure (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Cadmium (Cd), known as modern toxic metal, is one of the most toxic substances released to the environment through a wide range of industrial activities worldwide, as it has global commercial importance. It is considered a major health hazard due to its long biological half-life (about 10-30 years) (Fan et al., 2018; Wang et al., 2019). According to the International Agency for Research on Cancer (IARC), Cd is classified as a group I carcinogen (IARC, 1976). A classic example of the ecotoxicological significance of Cd is Itai-Itai disease, which is characterized by severe pain, bone fractures, proteinuria, and osteomalacia, predominantly among women. It is stated that this disease is caused by consumption of rice grown on soil contaminated with Cd and drinking contaminated water caused by a mining activity along with nutritional factors (Inaba et al., 2005; Nordberg, 2009; Ono, 2013). Since Cd is not biodegradable and has a long half-life, it accumulates in various tissues and organs such as testis, kidney, lung, liver, brain, heart and central nervous system. Oxidative tissue damage occurs with the formation of superoxide anion, hydroxyl radical and other reactive oxygen species (ROS). (Nwokocha et al., 2019). It is known that accelerating the accumulation of ROS by Cd increases oxidative stress and lipid peroxidation, consuming glutathione (GSH) and protein-bound thiol groups and triggers DNA damage (Banik et al., 2019; Kim et al., 2019; Zhao et al., 2019).

Oxidative stress is mainly caused by the imbalance between antioxidants and oxidants, which disrupts the structure of organs, cells and tissues in the human body. Oxidative stress has been shown to play an important role in the pathogenesis of various diseases such as cardiovascular disease and myocardial infarction, cardiomyopathy, ischemic stroke, hypertensive heart disease and metabolic disorders (Jing, Yan, Yuan, & Zhu, 2019). Excessive free radical production weakens the body's antioxidant defense mechanism and damages proteins, lipids, DNA and other biomolecules. Endogenous antioxidant defense mechanisms include enzymatic systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), as well as non-enzymatic agents such as ascorbic acid, alpha tocopherol and GSH (Pal et al., 2019).

Sildenafil (Sil), a phosphodiesterase type 5 (PDE-5) inhibitor, was originally developed for the treatment of angina pectoris, but has subsequently been extensively used clinically in the treatment of erectile dysfunction and pulmonary hypertension (Lombardi, Nelli, Celso, Mencarini, & Del Popolo, 2012). Sil is a selective PDE-5 inhibitor leading to accumulation of cyclic guanosine monophosphate (cGMP). With this mechanism, it increases the effects of endogenous nitric oxide (NO), leading to penile smooth muscle relaxation, thereby creating arterial dilatation, which leads to the expansion of the corpus cavernosum (de Carvalho et al., 2019; Venkat et al., 2019). Many recent studies have shown the effectiveness of Sil in preventing organ damage due to oxidative stress and inflammation (Abdel-latif, Morsy, El-Moselhy, & Khalifa, 2013; Kaur et al., 2017; Zahran et al., 2015). In another study, tadalafil, a PDE-5 inhibitor, has been shown to improve peripheral vascular insufficiency and reduce peripheral neuropathy in mice (Ogihara et al., 2019). Tadalafil administration in individuals with type 2 diabetes mellitus has been reported to promote improvement in beta cell function in metabolic syndrome independent of insulin sensitivity in both men and women. (Hill, Eckhauser, Marney, & Brown, 2009).

In this study, we aimed to investigate the different pharmacological activities of Sil, which is widely used in pulmonary hypertension and erectile dysfunction in the clinic, to evaluate the benefits of organ and tissue damage in different organs due to exposure to heavy metal such as Cd, furthermore, to contribute to the studies conducted to prevent organ damage through the data and experience we provide.

MATERIALS AND METHODS

Experimental animals and ethics committee

In this study, 28 Sprague-Dawley female rats obtained from Fırat University Experimental Animals Production and Research Center were used. The experimental protocols of the study were approved by Fırat University Faculty of Medicine Animal Research Committee (Ethics committee protocol no: 2016/151) and the ethical rules were fully complied with in the study. Female rats weighing 250-300 grams were kept in standard housing cages until the day of the experiment. Routine maintenance services such as daily change of drinking water of animals, feeding of feed and standard cage cleaning were performed by Fırat University Experimental Animals Production and Research Center personnel. The rats were housed in rooms with dark / light illumination for 12 h, at room temperature of 24-27 °C with appropriate humidity and ventilation. Seven rats were housed in each cage and the rats were divided into 4 groups. The groups were designed with 7 rats in each group in order to ensure statistical significance by considering other studies.

Experimental groups;

- **Group 1** (Control, ad libitum feeding for 10 days, 0,5 ml/rat distilled water intraperitoneally (ip) on day 7, n = 7)
- **Group 2** (Cd, ad libitum feeding for 10 days, 3,7 mg/kg single dose Cd ip at day 7, n = 7)

- **Group 3** (Sil, ad libitum feeding for 10 days and 5 mg/kg /day Sil in drinking water, n = 7)
- **Group 4** (Cd + Sil, ad libitum feeding for 10 days, 5 mg/kg /day Sil in drinking water + 3,7 mg/kg single dose Cd ip at day 7, n = 7)

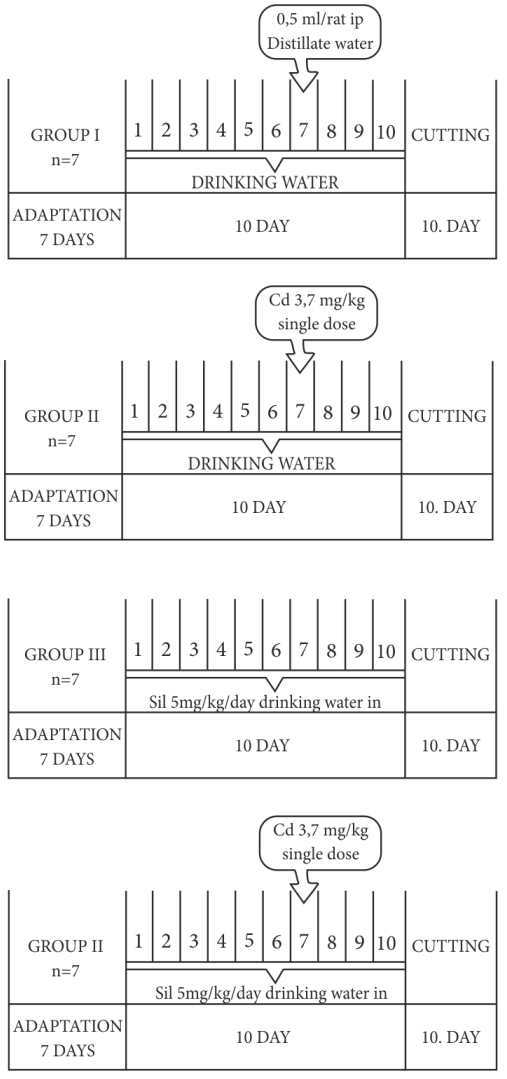

Figure 1. Schematic representation of the experimental design.

Experimental method

Sil citrate was purchased from Pfizer and $CdCl_2$ was purchased from Merck. $CdCl_2$ and Sil citrate were dissolved in distilled water, the injection volume adjusted to 0,5 ml / rat. On the 7th day, a single dose of 3,7 mg/kg Cd was injected ip in the morning and Sil was administered in drinking water for 10 days. On the 10th day of the experiment, 72 hours after Cd administration, the rats were sacrificed under anesthesia and the lungs, kidneys and livers of the rats were isolated and Thiobarbituric acid reactive substances (TBARs), GSH, total thiol (T-SH) levels in these tissues were evaluated. Tissue damage was assessed by serum alanine aminotransferase (ALT), aspartataminotransferase (AST), creatinine, blood urea nitrogen (BUN) and creatine kinase (CK-MB).

At the end of the experiment, 50 mg/kg ketamine and 5 mg/kg xylazine anesthesia were applied ip to the animals. Blood samples obtained from the heart of the rats were taken into gel tubes and centrifuged at 3000 rpm for 15 minutes. The lung, kidney and liver tissues of the rats were removed with scalpel. These tissues were immediately stored in locked bags at -20 ° C and then at -80 ° C until measurements were made.

Preparing tissues for measurement

Before the tissues were removed from the refrigerator at -80 ° C, a working environment with ice water was prepared in order to prevent the structure of sensitive molecules such as enzymes and hormones from deteriorating, and the whole study was carried out in this environment. Approximately 100 mg of lung, kidney and liver tissues were taken and weighed on a sensitive balance and placed in plastic tubes. After adding 1 ml of phosphate buffer onto the tubes, it was homogenized with a homogenizer, then sonification was performed, the supernatants obtained after centrifugation were taken into ependorphic tubes and used for the measurement of biochemical parameters.

AST, ALT, CREATINE, CK-MB and BUN measurement in serum

An auto analyzer was used to measure these parameters. Results were expressed as IU/L for AST, ALT and CK-MB and mg/dl for creatinine and BUN.

T-SH, TBARS, GSH measurement in tissue

For T-SH, TBARS and GSH determinations, measurements were performed by ELISA using commercial kits from Shanghai Yehua Biological Technolgy. The detailed detection method of each biochemical assay was performed according to the manufacturer's instructions.

Statistical analysis

Graph Pad Instat Version 3.10 package program was used for statistical analysis. Data are expressed as mean ± standard error (± SEM). One-way analysis of variance (ANOVA) was used to determine the difference between the groups. Multiple comparisons were made with Tukey-Kramer test. $p < 0.05$ was considered significant ($p < 0.05$: * significant, $p < 0.01$: ** very significant and $p < 0.001$: *** was considered extremely significant).

RESULTS

Serum AST, ALT, BUN, CK-MB and Creatinine levels were significantly increased in the Cd group compared to the control group (for all parameters; $p < 0.001$). Compared to the Cd group, serum AST, ALT, BUN, CK-MB and Creatinine levels were significantly reduced in the Sil + Cd group (AST, ALT; $p < 0.001$, Creatinine; $p < 0.001$, BUN; $p < 0.01$, CK-MB; $p < 0.05$) (Figure 2).

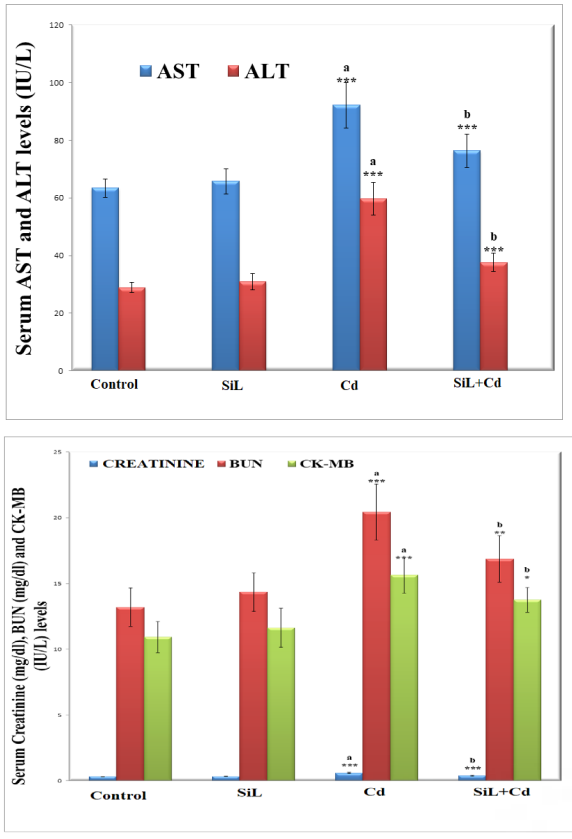

Figure 2. Serum AST, ALT, BUN, Creatinine and CK-MB levels, * p<0.05, **p<0.01, ***p<0.001 (n=7, ANOVA)

AST and ALT ;

a; According to the control group; An extremely significant increase was found in the Cd group (p<0.001).

b; According to the cadmium group; A significant decrease was observed in the Sil+Cd group (p<0.001).

Creatinine, BUN and CK-MB ;

a; According to the control group; An extremely significant increase was found in the Cd group (p<0.001).

b; According to the cadmium group; A significant decrease was observed in the Sil+Cd group (Creatinine; p<0.001, BUN; p<0.01, CK-MB; p<0.05).

Cd administration caused a significant increase in tissue TBARS levels in all tissues (p <0.001). Compared to Cd group, tissue TBARS levels significantly decreased in Sil + Cd group (p <0.001). Compared to the control group, tissue GSH levels showed a significant decrease in all tissues in the Cd group (p <0.001). The decrease in GSH caused by Cd was significantly prevented by the Sil administration in the Sil + Cd group compared to the Cd group (p <0.05). T-SH levels were significantly decreased in the Cd group compared to the control group (p <0.001). The decrease in tissue T-SH levels caused by Cd significantly prevented by Sil administration in liver and kidney tissues (p <0.01), while there was no significant difference in lung tissue (Figure 3).

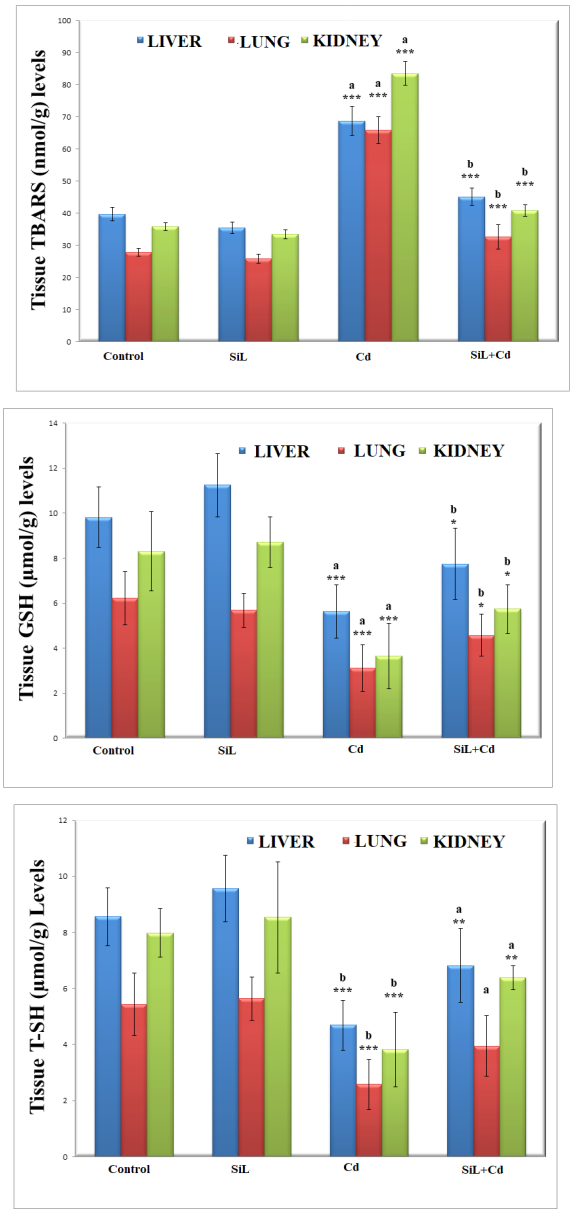

Figure 3. Tissue TBARS, GSH, T-SH levels. * p<0.05, **p<0.01, *** p<0.001 (n=7, ANOVA)

TBARS;

a; According to the control group; Cd application caused a very significant increase in all tissues (p<0.001).

b; According to the cadmium group; Significant decreases were observed in the Sil+Cd group (p<0.001).

GSH;

a; According to the control group; A very significant reduction in all tissues was detected in the Cd group (p<0.001).

b; According to the cadmium group; In the Sil+Cd group, Sil application significantly prevented the decrease of GSH caused by Cd (p<0.05).

T-SH;

a; According to the control group; A very significant decrease was found in the Cd group (p<0.001).

b; According to the cadmium group; Sil application has been shown to significantly prevent the decrease caused by Cd in liver and kidney (p <0.01), no significant difference was found in the lung.

DISCUSSION

In this study, in which we investigated the effect of Sil against Cd-induced multiple organ damage in rats in the light of biochemical and oxidative stress parameters, supporting the previous studies, Cd application caused significant damage to liver lung and kidney tissue ($p < 0.001$). TBARS, a low molecular weight lipid peroxidation byproduct, is mainly composed of malondialdehyde (MDA) with ROS attack in membrane phospholipids and is widely used as an oxidative stress marker. In our study, increased TBARS levels were found in the Cd group that was supported by previous studies compared to the control group (Massó, Corredor, & Antonio, 2007). Some studies have shown that Sil decreases MDA levels in spinal and testicular damage by increasing antioxidant activity (Beheshtian et al., 2008; Serarslan et al., 2010). Increased biochemical parameters as a sign of cellular damage due to oxidative stress and decreased GSH and T-SH levels, which play an important role in cellular defense, suggest that Cd causes oxidative damage at the cellular level. Cd is a dangerous and toxic heavy metal that causes serious harmful health problems in humans and animals. It causes cytotoxicity, carcinogenicity and mutagenicity in the organism and also triggers free radical production, causing oxidative damage to lipids, proteins and DNA (Saggu et al., 2019). When it is taken into the body, it quickly disperses throughout the body and triggers tissue damage through inflammatory and oxidative stress-based mechanisms. Cd is replaced by bivalent cations in the active sites of different enzymes, including antioxidant enzymes, leading to disruption of cellular redox homeostasis (Salama, Arab, Hassan, & Maghrabi, 2019). The exposed Cd accumulates most intensely in the liver and kidneys and causes nephrotoxicity. When Cd is taken into kidney cells, cellular free radical production accelerates and apoptosis occurs (Seif, 2019). The liver is the main target organ of Cd toxicity (Baba et al., 2013). Some studies have reported the use of different chelating agents to alleviate Cd toxicity by increasing Cd excretion; however, chelation therapy is controversial in terms of efficiency and safety (Kojima et al., 1992). Cd shows high affinity for compounds containing thiol group. Other compounds carrying a reduced GSH and thiol group are very critical compounds that play a key role in the antioxidant defense system. This is because the depletion of thiols greatly contributes to the tissue damage caused by ROS (Singhal, Anderson, & Meister, 1987). In a study investigating the antioxidant effect of *Cabbage (Brassica oleracea var. capitata)* plant in Cd-induced oxidative damage, it was reported that Cd administration produced a significant decrease in liver and lung lipid peroxidation levels as well as liver GSH levels (Eryılmaz, Deliorman Orhan, Aktay, & Bingöl, 2002). In our study, while lipid peroxidation increased with Cd administration, decrease in GSH and T-SH levels was found to be compatible with the literature.

Liver enzymes (AST, ALT) are widely used in the studies related to liver as the most specific indicator of liver damage. (Saggu et al., 2019). In the Cd group of our study, it was found that there was a significant ($P < 0.001$) increase in serum AST and ALT enzyme levels compared to the control group. These results are consistent with previous studies showing impaired liver function with Cd (Almeer et al., 2018).

The data obtained from the study showed that administration of a single dose of Cd at a dose of 7.5 mg/kg produced acute kidney toxicity in rats and changes in kidney function were characterized by increases in BUN and serum creatinine concentrations.

In our study, we observed that healing occurred in nearly all tissues in the group where we applied Sil+Cd, close to the control group. The ROS removal ability detected by the Sil application is similar to the free radical scavenging effect of well-known antioxidants such as N-acetylcysteine, GSH and CAT (Luanpitpong et al., 2013). Our results show the antioxidant potential of Sil in lung, liver and kidney toxicity. Another study reported the antioxidant and anti-inflammatory effect of Sil against acute lung injury due to burns in rats (Gokakin et al., 2013). PDE-5 inhibitors seem promising, especially in the treatment of lung damage, because their PDE-5 activities are greatly enhanced in oxidative stress and inflammatory processes. In a study performed by Shimaa El-Metwaly et al. in hydrochloric acid-induced lung injury, Sil was shown to provide a significant decrease in MDA levels and a significant increase in GSH levels in lung tissue (El-Metwaly, El-Senduny, EL-Demerdash, & Abdel-Aziz, 2019). In a study conducted in rats, where the effect of Sil and febuxostat on doxorubicin nephrotoxicity was investigated, while serum urea, creatinine and uric acid levels were used as nephrotoxicity biomarkers; GSH and MDA levels were used as oxidative stress biomarkers and it was observed that nephrotoxicity and oxidative stress markers showed significant improvement with Sil administration (Khames, Gad, & El-Raouf, 2017). Cisplatin is an antineoplastic agent that can cause kidney damage with many different mechanisms. In a study in which cisplatin was applied to rats, Sil was reported to be able to alleviate kidney damage by clearing free oxygen radicals (Lee et al., 2009). In a different study by Ahmed A. Elberry et al., the role of Sil as a curative was investigated in cisplatin-induced rat nephrotoxic model, it was reported that Sil treatment could protect rats from cisplatin-induced nephrotoxicity by improving glomerular filtration rate and kidney blood flow. These healing effects may partly be attributed to their antioxidant and anti-inflammatory effects. (Elberry & Almohamadi, 2014).

Some studies have reported that Sil can produce hepatotoxic syndrome, in contrast to the data we obtained in our study. In a case study, it was stated that

a patient who had been using 50 mg tablet Sil several times in the 3 months prior to illness, probably had drug-related hepatotoxic syndrome (Wolfhagen, Vermeulen, Rob, & Lesterhuis, 2008). In the study conducted by Mofrih M Hegazy et al., rats were given as 26 mg/kg/day for 28 days Sil citrate via oral gavage, and necrosis was observed in hepatocytes with a significant increase in the serum damage markers AST and ALT serum levels. (Hegazy, Elhalfawy, Shaban, & Abouelnour, 2018). In the same study, degenerative and atrophic changes were observed in the kidneys in the group treated with Sil and were associated with higher urea and creatinine levels indicating nephrotoxicity. These results are most likely related to the administration of Sil at a higher dose (26 mg/kg) than the dose administered in our study.

Wesam El-Bakly et al. investigated the efficacy and underlying mechanism of PDE-5 inhibitors in cognitive impairment, and they have reported that the PDE-5 inhibitors may help prevent cognitive impairment in Alzheimer's disease. It is stated that this effect is likely to be accomplished by improving apoptosis and inflammation processes together with antioxidant activity (Bakly et al., 2019).

CONCLUSION

Recent developments show that the environmental level of heavy metals increases with human activities. Acute and chronic exposures to heavy metals seriously threaten the health of all living organisms. Therefore, toxicity studies from heavy metals will become more and more important. Due to the discovery of new drugs and clinical trials are long term, challenging and quite costly, the researchers have tended to investigate alternative pharmacological activities of current drugs in treatment. Sil, which is a pharmacologically important drug in current treatment, may have an alternative pharmacological function in treating heavy metal oxidative damage. As a result of the study in which we examined Sil's mechanism of action, we think that Sil can be protective against multiple organ damage caused by heavy metals, and this effect is likely to activate the antioxidant defense system. However, this beneficial effect should be supported by further clinical studies.

Acknowledgements

The authors would like to thank Mrs Ayşe Burçin Uyumlu for supporting ELISA studies. The parameters evaluated in this study were published so as not to lose their validity. This issue will continue to be studied with different parameters in subsequent studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

Abdel-latif, R. G., Morsy, M. A., El-Moselhy, M. A., & Khalifa, M. A. (2013). Sildenafil protects against nitric oxide deficiency-related nephrotoxicity in cyclosporine A treated rats. *European Journal of Pharmacology, 705*(1-3), 126-134. doi: 10.1016/j.ejphar.2013.02.039

Almeer, R. S., Alarifi, S., Alkahtani, S., Ibrahim, S. R., Ali, D., & Moneim, A. (2018). The potential hepatoprotective effect of royal jelly against cadmium chloride-induced hepatotoxicity in mice is mediated by suppression of oxidative stress and upregulation of Nrf2 expression. *Biomedicine & Pharmacotherapy, 106*, 1490-1498. doi: 10.1016/j.biopha.2018.07.089

Baba, H., Tsuneyama, K., Yazaki, M., Nagata, K., Minamisaka, T., Tsuda, T., . . . Nakajima, T. (2013). The liver in itai-itai disease (chronic cadmium poisoning): pathological features and metallothionein expression. *Modern Pathology, 26*(9), 1228. doi: 10.1038/modpathol.2013.62

Bakly, W. E., Wagdy, O., Sobhy, A., Riad, M. S., El Sayed, M., Tarkhan, S., . . . Nabil, N. (2019). The Efficacy and Underlying Mechanism of Phosphodiesterase-5 inhibitors in Preventing Cognitive Impairment and Alzheimer Pathology: A Systematic Review of Animal Studies. *Behavioural Brain Research*, 112004. doi: 10.1016/j.bbr.2019.112004

Banik, S., Akter, M., Bondad, S. E. C., Saito, T., Hosokawa, T., & Kurasaki, M. (2019). Carvacrol inhibits cadmium toxicity through combating against caspase dependent/independent apoptosis in PC12 cells. *Food and Chemical Toxicology, 134*, 110835. doi: 10.1016/j.fct.2019.110835

Beheshtian, A., Salmasi, A. H., Payabvash, S., Kiumehr, S., Ghazinezami, B., Rahimpour, S., . . . Dehpour, A. R. (2008). Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. *World Journal of Urology, 26*(2), 197-202. doi: 10.1007/s00345-008-0243-6

de Carvalho, M. A. J., Chaves-Filho, A., de Souza, A. G., de Carvalho Lima, C. N., de Lima, K. A., Vasconcelos, E. R. R., . . . Macedo, D. S. (2019). Proconvulsant effects of sildenafil citrate on pilocarpine-induced seizures: Involvement of cholinergic, nitrergic and pro-oxidant mechanisms. *Brain Research Bulletin, 149*, 60-74. doi: 10.1016/j.brainresbull.2019.04.008.

El-Metwaly, S., El-Senduny, F. F., EL-Demerdash, R. S., & Abdel-Aziz, A. (2019). Mesenchymal stem cells alleviate hydrochloric acid-induced lung injury through suppression of inflammation, oxidative stress and apoptosis in comparison to moxifloxacin and sildenafil. *Heliyon, 5*(12), e02710. doi: 10.1016/j.heliyon. 2019.e02710

Elberry, A. A., & Almohamadi, A. M. (2014). Sildenafil ameliorates cisplatin-induced nephrotoxicity in wistar rats. *World J Pharm Pharm Sci, 3*, 151-161. https://pdfs.semanticscholar.org/e395/ef01440c07b731bf7d73f16e920bf1005763.pdf?_ga=2.98785502.1397669013.1581413979-1800345763.1574856951

Eryılmaz, B., Deliorman Orhan, D., Aktay, G., & Bingöl, F. (2002). Antioxidant effect of cabbage (brassica oleracea var. capitata) on oxidative stress in cadmium treated mice. *FABAD Journal of Pharmaceutical Sciences, 27*(1), 1-5. http://dergi.fabad.org.tr/pdf/volum27/Issue1/1.pdf

Fan, R., Hu, P. C., Wang, Y., Lin, H. Y., Su, K., Feng, X. S., . . . Yang, F. (2018). Betulinic acid protects mice from cadmium chloride-induced toxicity by inhibiting cadmium-induced apoptosis in kidney and liver. *Toxicology Letters, 299*, 56-66. doi: 10.1016/j.toxlet.2018.09.003

Gokakin, A. K., Deveci, K., Kurt, A., Karakus, B. C., Duger, C., Tuzcu, M., & Topcu, O. (2013). The protective effects of sildenafil in acute lung injury in a rat model of severe scald burn: a biochemical and histopathological study. *Burns, 39*(6), 1193-1199. doi: 10.1016/j.burns.2012.12.017

Hegazy, M., Elhalfawy, I., Shaban, M., & Abouelnour, E. (2018). Potential Protective Role of Nigella sativa Linn against Sildenafil Induced Hepatotoxicity and Nephrotoxicity in Adult Male Rabbits. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 30*(1), 101-109. doi: 10.21608/AJFM.2018.18200

Hill, K. D., Eckhauser, A. W., Marney, A., & Brown, N. J. (2009). Phosphodiesterase 5 inhibition improves β-cell function in metabolic syndrome. *Diabetes Care, 32*(5), 857-859. doi: 10.2337/dc08-1862

IARC. (1976). *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics* (Vol. 11). https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Cadmium-Nickel-Some-Epoxides-Miscellaneous-Industrial-Chemicals-And-General-Considerations-On-Volatile-Anaesthetics-1976

Inaba, T., Kobayashi, E., Suwazono, Y., Uetani, M., Oishi, M., Nakagawa, H., & Nogawa, K. (2005). Estimation of cumulative cadmium intake causing Itai–itai disease. *Toxicology Letters, 159*(2), 192-201. doi: 10.1016/j.toxlet.2005.05.011

Jing, C., Yan, C.-J., Yuan, X.-T., & Zhu, L.-P. (2019). Biosynthesis of copper oxide nanoparticles and their potential synergistic effect on alloxan induced oxidative stress conditions during cardiac injury in Sprague–Dawley rats. *Journal of Photochemistry and Photobiology B: Biology, 198*, 111557. doi: 10.1016/j.jphotobiol.2019.111557

Kaur, M., Singh, A., Kumar, B., Singh, S. K., Bhatia, A., Gulati, M., . . . Malik, A. H. (2017). Protective effect of co-administration of curcumin and sildenafil in alcohol induced neuropathy in rats. *European Journal of Pharmacology, 805*, 58-66. doi: 10.1016/j.ejphar.2017.03.012

Khames, A., Gad, A. M., & El-Raouf, O. M. A. (2017). Ameliorative effects of sildenafil and/or febuxostat on doxorubicin-induced nephrotoxicity in rats. *European Journal of Pharmacology, 805*, 118-124. doi: 10.1016/j.ejphar.2017.02.046

Kim, S. S., Meeker, J. D., Keil, A. P., Aung, M. T., Bommarito, P. A., Cantonwine, D. E., . . . Ferguson, K. K. (2019). Exposure to 17 trace metals in pregnancy and associations with urinary oxidative stress biomarkers. *Environmental Research, 179*, 108854. doi: 10.1016/j.envres.2019.108854

Kojima, S., Sugimura, Y., Hirukawa, H., Kiyozumi, M., Shimada, H., & Funakoshi, T. (1992). Effects of dithiocarbamates on testicular toxicity in rats caused by acute exposure to cadmium. *Toxicology and Applied Pharmacology, 116*(1), 24-29. doi: 10.1016/0041-008X (92)90140-N

Lee, K. W., Jeong, J. Y., Lim, B. J., Chang, Y.-K., Lee, S.-J., Na, K.-R., . . . Choi, D. E. (2009). Sildenafil attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. *Toxicology, 257*(3), 137-143. doi: 10.1016/j.tox.2008.12.017

Lombardi, G., Nelli, F., Celso, M., Mencarini, M., & Del Popolo, G. (2012). Treating erectile dysfunction and central neurological diseases with oral phosphodiesterase type 5 inhibitors. Review of the literature. *The Journal of Sexual Medicine, 9*(4), 970-985. doi: 10.1111/j.1743-6109.2011.02615.x

Luanpitpong, S., Chanvorachote, P., Stehlik, C., Tse, W., Callery, P. S., Wang, L., & Rojanasakul, Y. (2013). Regulation of apoptosis by Bcl-2 cysteine oxidation in human lung epithelial cells. *Molecular Biology of The Cell, 24*(6), 858-869. doi: 10.1091/mbc.E12-10-0747

Massó, E. L., Corredor, L., & Antonio, M. T. (2007). Oxidative damage in liver after perinatal intoxication with lead and/or cadmium. *Journal of Trace Elements in Medicine and Biology, 21*(3), 210-216. doi: 10.1016/j.jtemb.2007.03.002

Nordberg, G. F. (2009). Historical perspectives on cadmium toxicology. *Toxicology and Applied Pharmacology, 238*(3), 192-200. doi: 10.1016/j.taap.2009.03.015

Nwokocha, C. R., Palacios, J., Rattray, V. R., McCalla, G., Nwokocha, M., & McGrowder, D. (2019). Protective effects of Apocynin against Cadmium toxicity and serum parameters; evidence of a cardio-protective influence. *Inorganica Chimica Acta*, 119411. doi: 10.1016/j.ica.2019.119411

Ogihara, T., Nakagawa, T., Hayashi, M., Koyanagi, M., Yonezawa, A., Omura, T., . . . Matsubara, K. (2019). Improvement of peripheral vascular impairment by a phosphodiesterase type 5 inhibitor tadalafil prevents oxaliplatin-induced peripheral neuropathy in mice. *Journal of Pharmacological Sciences, 141*(4), 131-138. doi: 10.1016/j.jphs.2019.10.005

Ono, K. (2013). Past and future cadmium emissions from municipal solid-waste incinerators in Japan for the assessment of cadmium control policy. *Journal of Hazardous Materials, 262*, 741-747. doi: 10.1016/j.jhazmat.2013.09.033

Pal, G., Behl, T., Rohil, V., Khandelwal, M., Gupta, G., & Jena, J. (2019). Evaluation of oxidative stress and its modulation by L-Arginine and L-Ascorbic acid in repetitive restraint stress model in Wistar rats. *Obesity Medicine*, 100172. doi: 10.1016/j.obmed.2019.100172

Saggu, S., Rehman, H., Alzeibr, F. M., Oyouni, A. A. A., Zidan, N., Panneerselvam, C., & Trivedi, S. (2019). Cymbopogon schoenanthus (Ethkher) ameliorates cadmium induced toxicity in swiss albino mice. *Saudi journal of biological sciences, 26*(7), 1875-1881. doi: 10.1016/j.sjbs.2017.01.002

Salama, S. A., Arab, H. H., Hassan, M. H., & Maghrabi, I. A. (2019). Cadmium-induced hepatocellular injury: Modulatory effects of γ-glutamyl cysteine on the biomarkers of inflammation, DNA damage, and apoptotic cell death. *Journal of Trace Elements in Medicine and Biology, 52*, 74-82. doi: 10.1016/j.jtemb.2018.12.003

Seif, M. (2019). Hepato-renal protective effects of egyptian purslane extract against experimental cadmium toxicity in rats with special emphasis on the functional and histopathological changes. *Toxicology Reports*. doi: 10.1016/j.toxrep.2019.06.013

Serarslan, Y., Yönden, Z., Özgiray, E., Oktar, S., Güven, E. O., Söğüt, S., . . . Yurtseven, T. (2010). Protective effects of tadalafil on experimental spinal cord injury in rats. *Journal of Clinical Neuroscience, 17*(3), 349-352. doi: 10.1016/j.jocn.2009.03.036

Singhal, R. K., Anderson, M. E., & Meister, A. (1987). Glutathione, a first line of defense against cadmium toxicity. *The FASEB Journal, 1*(3), 220-223. doi: 10.1096/fasebj.1.3.2887478

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. In *Molecular, Clinical and Environmental Toxicology* (pp. 133-164): Springer. doi: 10.1007/978-3-7643-8340-4_6

Venkat, P., Chopp, M., Zacharek, A., Cui, C., Landschoot-Ward, J., Qian, Y., . . . Chen, J. (2019). Sildenafil treatment of vascular dementia in aged rats. *Neurochemistry International, 127*, 103-112. doi: 10.1016/j.neuint.2018.12.015

Wang, L., Zheng, M., Zhang, S., Zhao, C., Kang, W., & Wang, K. (2019). Roles of mtDNA damage and disordered Ca (2+) homeostasis in the joint toxicities of cadmium and BDE209. *Ecotoxicology and Environmental Safety, 186*, 109767. doi: 10.1016/j.ecoenv.2019.109767

Wolfhagen, F. H., Vermeulen, H. G., Rob, A., & Lesterhuis, W. (2008). Initially obscure hepatotoxicity attributed to sildenafil. *European Journal of Gastroenterology & Hepatology, 20*(7), 710-712. doi: 10.1097/MEG.0b013e3282f2bbb5

Zahran, M. H., Hussein, A. M., Barakat, N., Awadalla, A., Khater, S., Harraz, A., & Shokeir, A. A. (2015). Sildenafil activates antioxidant and antiapoptotic genes and inhibits proinflammatory cytokine genes in a rat model of renal ischemia/reperfusion injury. *International Urology and Nephrology, 47*(11), 1907-1915. doi: 10.1007/s11255-015-1099-5

Zhao, Q., Yang, Z.-S., Cao, S.-J., Chang, Y.-F., Cao, Y.-Q., Li, J.-B., . . . Wu, R. (2019). Acute oral toxicity test and assessment of combined toxicity of cadmium and aflatoxin B1 in kunming mice. *Food and Chemical Toxicology*, 110577. doi: 10.1016/j.fct.2019.110577