



Effects of Different Doses of Simvastatin on Lead-Induced Kidney Damage in Balb/C Male Mice

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ABSTRACT

Background: Lead is known to be a highly toxic heavy metal. There are a limited number of studies investigating the effects of antioxidants on lead-induced kidney damage. Statins are widely used drugs for the treatment of hypercholesterolaemia, but they also have other pleiotropic effects. The aim of this study was to determine the effect of different doses of simvastatin on biochemical and histopathological parameters in mice exposed to lead. **Methods:** Forty eight adult male mice were randomised into six groups. The control group received no lead. Group II was injected intraperitoneally with 60 mg/kg lead acetate and groups III-VI received intraperitoneally 5-10-20-40 mg/kg simvastatin plus 60 mg/kg lead. After 14 days, 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 SPSS software and ANOVA. **Results:** Lead acetate treatment caused collapse of glomeruli, glomerulosclerosis, necrosis and vacuolization in renal tubules. Administration of 20 mg of simvastatin reduced the severity of kidney damage. Glomerular volume in the groups treated with 40 mg of simvastatin was significantly different from the group treated with lead alone ($P=0.001$). The number of renal glomeruli in the group treated with 5 mg of simvastatin were significantly difference compared to the lead treated group ($P=0.027$). Serum concentrations of urea and creatinine were not significantly different in the groups treated with simvastatin compared to the group treated with lead alone. **Conclusions:** Treatments with simvastatin Caused protective effects on renal tissue of mice exposed to lead. However, there was no significant effect on urea and creatinine levels.

Introduction

Lead, one of the most important environmental pollutants, is widely used in the chemical industry and it has been a major problem for human health.¹ Despite the removal of lead from gasoline in many countries, lead levels in the environment are much higher than those recommended by the World Health Organization.² The occupational exposure upper limit for lead has been recommended to be $<150 \mu\text{g}/\text{m}^3$ for 8-hour time. The non-occupational exposure limit is the

range between $26\text{-}282 \mu\text{g}/\text{day}$.³ Lead accumulates at very high levels in the kidneys and causes structural histopathological changes and functional changes of the kidney.⁴ Animal studies suggest that lead causes reversible effects in the kidney, such as cytomegali and inclusion body in the nucleus and irreversible symptoms such as interstitial fibrosis and chronic renal failure.⁴⁻⁶ Lead poisoning causes a reduction in glomerular volume but an increase in number of glomeruli.⁷ After lead administration, kidney shrinkage,

elimination of the glomeruli and mild fibrosis with reduction in cortical thickness is observed. Atrophy and dilation are found in many renal tubules.⁸

Statins are commonly prescribed for lowering serum cholesterol levels.^{9,10} In addition, they have antioxidant and anti-inflammatory properties.¹¹ Several studies have shown that statins such as simvastatin causes protective effects in tissue damage, and has been shown to be protective agent against hepatic and renal toxicity induced by cisplatin in rats.¹² Also, simvastatin reduced the extent of renal lesions in a mouse model of chronic renal failure.¹³ Other studies have shown that simvastatin can reduce the damage caused by oxidative stress and improves proteinuria and glomerular damage in rats with nephrotic syndrome,¹⁴ and that simvastatin has antioxidant effects in renal tissue after injury.¹⁵⁻¹⁸ As far as we are aware, there is a little data on the effects of simvastatin on the effects of lead related renal disease. Hence, the aim of this study was to determine the effect of different doses of simvastatin on the lead poisoning in adult male mice.

Materials and Methods

After approval by the ethics committee, this study was conducted in 48 male Balb/c mice. The mice were kept in the standard 12-hour darkness, brightness and temperature 21-23°C. In addition, food and water were freely available. Mice were randomised to 6 groups containing eight mice as follows: a control group, a lead group and experimental III-VI groups. The control group received no injections. Group II was injected with 60 mg/kg lead acetate (manufactured by Sigma) and groups III-VI received intraperitoneally 5-10-20-40 mg/kg simvastatin plus 60 mg/kg lead acetate for 14 days. The appropriate dose of lead acetate was chosen based on the mortality of mice. To determine the optimal dose of lead acetate, it was injected different doses of lead acetate. Many mice died in the first week at high doses such as 120 mg/kg. So, the dose of 60

mg/kg lead acetate was used for study.

Stereological study

After 14 days, the mice were anesthetized with chloroform and kidneys were removed and placed into 10% fixative solution. Dehydration and clearing were performed by passing through ethanol 70%-100% and xylene. After the paraffin embedding, samples were sectioned using microtome with 5m thickness and stained with H&E. The stained slides were examined by

Olympus BX51 light microscopy.¹⁹

To calculate the total volume of the kidney, cortex, medulla and renal glomeruli was used the principle of Cavalier. The sections were randomly selected and a spot probe was randomly placed on the pictures. Then, the number of points hitting the kidney transects were counted. Following formula was used to calculate the volume of the cortex, medulla and kidney.

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

$\sum \pi$: total points of grid hitting the cortex, medulla and kidney, $a(p)$: 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:

$$V_{\text{total}} (\text{Glomeruli}) = V_v (\text{Glomeruli}) \times V (\text{reference})$$

Eq.(2)

Biochemical analysis

Auto analyzer and pars tous kits were used for measurement of serum levels of urea and creatinine.

Statistical analysis

Data are presented as mean $\pm$ SD. Then, data were analyzed using SPSS software version 20 and ANOVA test. Turkey was used for post-hoc test. A significant difference was accepted for a $P < 0.05$.

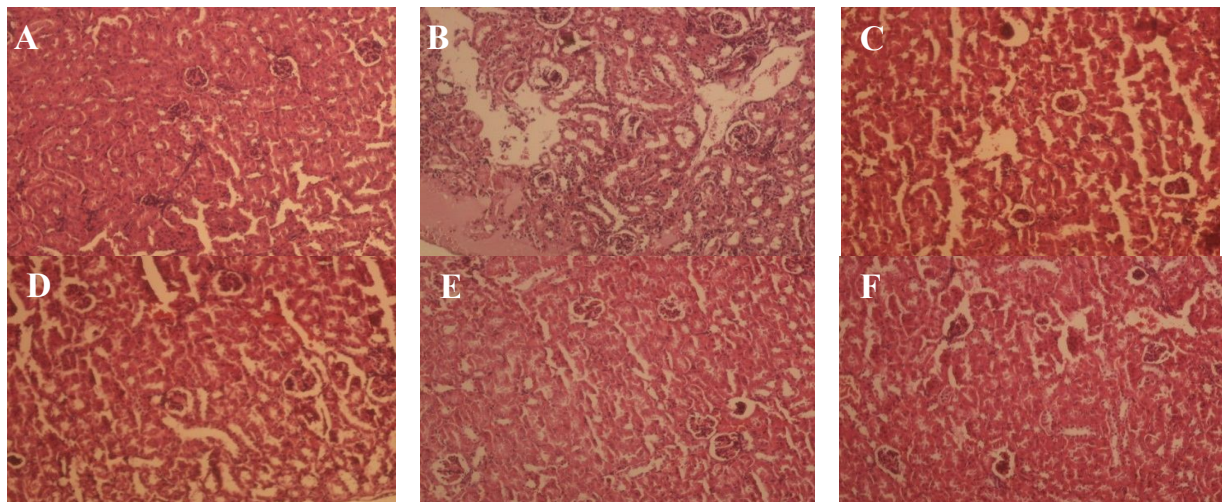


Figure 1. (A-F) are images of renal glomeruli in the control group, lead group, 5 mg/kg simvastatin group, 10 mg/kg simvastatin group, 20 mg/kg simvastatin group and 40 mg/kg simvastatin group. Magnification $\times 20$, H&E staining.

Histopatological results

For the control samples, glomeruli and tubules were normal and no pathological damage was observed. In addition, interstitial and renal arteries were also normal. In the present study after administration of lead acetate, glomeruli were collapsed and there was the presence of glomerulosclerosis. In addition, necrosis, cast and vacuolization were observed in convoluted tubules. In some samples, the congestion in the interstitial tissue, blood vessels as well as mild focal inflammation were observed (figure 1, 2). In the group treated with 5 mg/kg simvastatin, glomeruli were collapsed and there was vacuolization, necrosis and cast were observed in

proximal convoluted tubules as well as the congestion in the interstitial tissue. In groups treated with 10 mg/kg simvastatin glomeruli were collapsed and vacuolization, necrosis and low level of cast were observed in proximal convoluted tubules. In groups treated with 20 mg/kg simvastatin, glomeruli were normal. Renal convoluted tubules were without cast with low level of vacuolization. In groups treated with 40 mg/kg simvastatin, glomeruli were collapsed and vacuolization and necrosis was observed in proximal convoluted tubules. The interstitial tissue was normal and the congestion was found in the blood vessels of kidney (figure 1, 2).

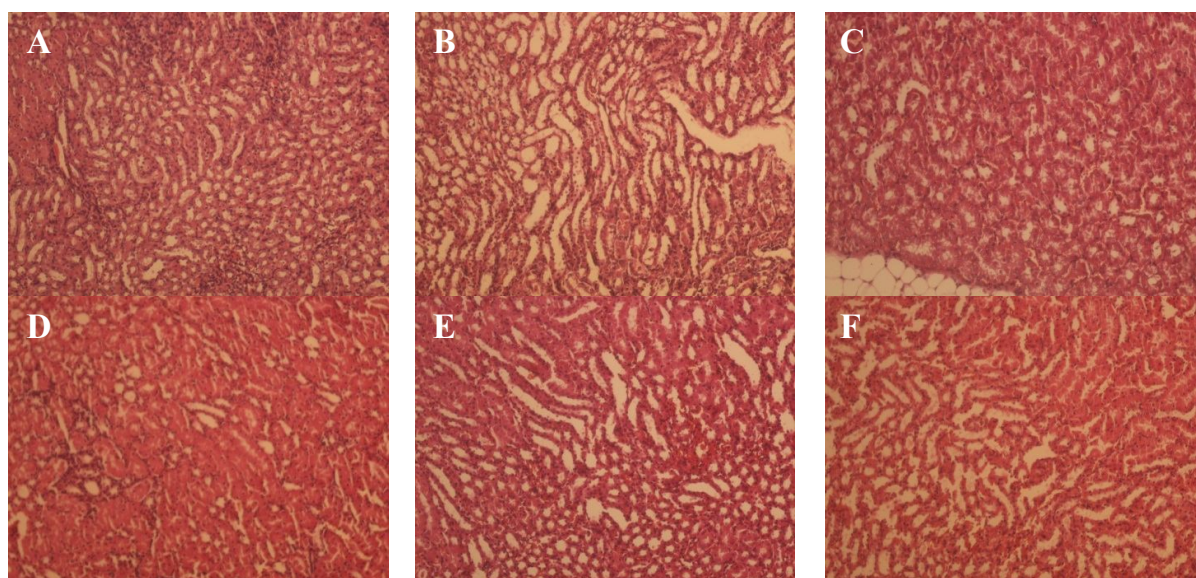


Figure 2. (A-F) are images of renal tubular in the control group, lead group, 5 mg/kg simvastatin group, 10 mg/kg simvastatin group, 20 mg/kg simvastatin group and 40 mg/kg simvastatin group. Magnification $\times 20$, H&E staining.

Kidney weight in experimental groups

Kidney weight to body weight ratio in the control group was 0.73 ± 0.07 that this ratio was 0.93 ± 0.08 for lead group. The statistical analysis showed a significant difference between the value of kidney ratio in the lead group compared to the control group ($P = 0.000$). In addition, the ratio of kidney weight to body weight in the groups treated with doses of 20 mg of simvastatin

significantly increased compared to the control group ($P=0.001$). There was a significant difference between values of kidney weight in group IV compared to these values of group V ($P=0.022$). Also, There was a significant difference between values of kidney weight in group III compared to these values of group IV ($P=0.007$) and group VI ($P=0.022$).

Table 1. Volume of different parts of kidney in experimental groups.

Experimental groups	Cortex volume (mm ³)	Medulla volume (mm ³)	Kidney volume (mm ³)	Glomerular volume (mm ³)	Glomerular count (mm ³)
Control group	4.81 $\pm$ 184.5	3.20 $\pm$ 287	1.60 $\pm$ 471.5	0.48 $\pm$ 4.88	777.4 $\pm$ 32013
Lead group	4.53 $\pm$ 144	10 $\pm$ 185.7	11.81 $\pm$ 329.7	0.52 $\pm$ 3.99	757.3 $\pm$ 22025
5 mg/kg simvastatin	5.67 $\pm$ 217.7	7.18 $\pm$ 190.2	12.85 $\pm$ 408	0.07 $\pm$ 4.60	794.1 $\pm$ 24025
10 mg/kg simvastatin	7.97 $\pm$ 180.7	5.67 $\pm$ 192.2	2.50 $\pm$ 373	0.19 $\pm$ 5.77	1440.9 $\pm$ 21075
20 mg/kg simvastatin	7.30 $\pm$ 203.5	5.67 $\pm$ 207.7	10.72 $\pm$ 411.25	0.23 $\pm$ 5.85	2155.8 $\pm$ 22075
40 mg/kg simvastatin	3.49 $\pm$ 180.2	6.07 $\pm$ 238.5	8.66 $\pm$ 418.7	0.06 $\pm$ 5.61	833.2 $\pm$ 26050

Cortex volume and medulla in experimental groups

Data of stereological study are summarized in Table 1. The statistical analysis showed significant differences between values of cortex and medulla volumes in the lead group compared to the control group ($P = 0.000$). Also, value of cortex volume in groups treated with 5 and 20 mg/kg simvastatin was significantly difference compared to the control groups ($P = 0.000$). There was a significant difference between values of cortex volume in group III compared to these values of group V ($P=0.001$). Medulla volume was significantly difference in groups treated with 20 and 40 mg/kg simvastatin compared to this value of the lead group ($P = 0.000$).

Volume and count of glomeruli in experimental groups

The statistical analysis showed a significant difference between values of Volume and count of glomeruli in the lead group compared to the control group ($P = 0.000$). Besides, glomerular volume was significantly difference in groups treated with 40 mg/kg simvastatin compared to this value in the control group ($P=0.001$). There was a significant difference between values of glomeruli volume in group V compared to these values of group VI ($P=0.025$). Glomerular count was significantly difference in groups treated with 5 mg/kg simvastatin compared to this value in the lead group ($P=0.027$). There was a significant difference between values of glomeruli count in group III compared to these values of group IV ($P=0.001$), group V ($P=0.04$) and group VI ($P=0.03$).

Level of urea and creatinine in the experimental groups

Data of serum urea and creatinine are summarized in Table 2. There was a significant differences between values of serum urea and creatinine in the lead group compared to the control group ($P = 0.000$). Also, urea levels in groups treated with simvastatin were significantly difference compared to those values of the lead group ($P=0.000$).

Table 2. Levels of urea and creatinine in experimental groups.

Experimental groups	Urea level (mg/dl)	Creatinine level (mg/dl)
Control group	3.50±29.06	0.06±0.48
Lead group	1.73±57.51	0.02±0.76
5 mg/kg simvastatin	2.81±44.96	0.04±0.79
10 mg/kg simvastatin	4.92±46.94	0.06±0.73
20 mg/kg simvastatin	5.08±42.26	0.08±0.72
40 mg/kg simvastatin	3.66±35.81	0.07±0.80

There was a significant difference between values of urea in group VI compared to these values of group III ($P=0.001$) and group V ($P=0.024$). It was not found a significant difference between values of the creatinine level in the treated groups compared to the lead group ($P>0.05$).

Discussion

Overall, the results of this study showed that lead acetate causes adverse effects on the kidney, particularly renal glomeruli. Besides, urea and creatinine, as an indicator of kidney function, dramatically increase after lead poisoning. Administration 20 mg/kg simvastatin can improve these effects. Studies have shown that lead poisoning causes a reduction in glomerular volume and an increase in number of glomeruli.⁷

After lead administration, kidney shrinkage, elimination of the glomeruli and mild fibrosis with reduction in cortical thickness is observed. Atrophy and dilation is found in many renal tubules.⁸ Another study showed that orally administration of lead caused renal damage, deposit of particles in the renal tubules and an increase in serum calcium level in rats.²⁰

Similarly, in the present study after administration of lead acetate, glomeruli were collapse and there was the presence of glomerulosclerosis. In addition, necrosis, cast and vacuolization were observed in convoluted tubules. In some samples, the congestion in the interstitial tissue, blood vessels as well as mild focal inflammation were observed in kidney.

Several studies have shown that statins such as simvastatin causes protective effects in tissue damage and has been shown to be protective agent against hepatic and renal toxicity induced by cisplatin in rats.¹² Also, simvastatin reduces the extent of renal lesions in mouse model of chronic renal failure.¹³ Other studies have shown that simvastatin can reduce the damage caused by oxidative stress and improves proteinuria and glomerular damage in the rats with nephrotic syndrome.¹⁴ Fouad et al reported that the administration of 5 mg/kg simvastatin significantly reduces the damage induced by cadmium. Also, expression of genes involved in the inflammatory response such as tumor necrosis factor- α , nuclear factor- κ B, cyclooxygenase-2, nitric oxide synthase and caspase-3 are reduced after simvastatin treatment. In addition, glutathione peroxidase and catalase levels increased while the levels of nitric oxide and malondialdehyde reduced in the treatment group compared to the non-treatment group. These researchers concluded that these effects are related to antioxidant and anti-inflammatory effects of simvastatin.²¹ Similarly to the above studies, in the current study administration of 20 mg simvastatin reduces tissue damage. Administration of other doses of simvastatin increased kidney damage that it is indicated a dose-dependent effect of simvastatin. The appropriate dose of simvastatin lead to the desired

effects while inappropriate doses of simvastatin causes destructive the effects.²²

Heilmann et al reported that an association between a reduction in the number of glomeruli and volume of glomeruli.²³ In consistent, in the present study, a reduction in the number of glomeruli in the kidney tissue and an increase in volume of glomeruli were observed. This may be compensated for the loss of functional glomeruli. In addition, our study showed that the lead administration increased kidney weight. Yagminas et al reported an increase in kidney weight following lead poisoning.²⁴ Increasing in kidney may be due to lead accumulation in the kidneys as well as an increasing inflammation and cell proliferative in the kidney.

Neshat's study showed that unilateral ureteral obstruction causes severe glomerular atrophy, subcapsular fibrosis, necrosis and atrophy in renal convoluted tubules. Serum urea and creatinine increase in this group. Prescription 2mg/ kg simvastatin for 15 days reduced the tissue damage caused by unilateral ureteral obstruction.²⁵ Also, in the present study, the dose of 20 mg of simvastatin reduced kidney damage but not effect on urea and creatinine levels.

It seems simvastatin due to antioxidant property has protective effect on renal and thereby neutralize free radicals and reduce oxidative stress. In addition, studies show anti-inflammatory properties of simvastatin. It reduces inflammatory markers such as interleukin and high sensitive C-reactive protein (hsCRP).^{9,11}

The strength of study was the investigation the effects of simvastatin on lead poisoning for the first time. The weaknesses of study were lack of evaluation the level of malondialdehyde, total antioxidant capacity and inflammatory factors that it recommends for future studies.

Conclusion

Histopathological results showed that lead acetate causes adverse effects on kidney tissue in mice. In addition, an increase in the kidney weight and the urea concentration was observed in the lead treated group. Administration 20 mg/kg simvastatin improved the histopathological induced by lead in mice during 14 days. However, there was no significant effect on urea and creatinine levels.

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