Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Original Article

Protective Effects of *Melissa officinalis* L. Extract on Gentamicin-induced Renal Failure in Diabetic Rats

Ali Reza Nasiri
(DVM) Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran,

Amir Reza Karamibonari
(PhD) Department of Pharmacology, Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran,

Corresponding author: Amir Reza Karamibonari

Tel: +989144172698

Email: pharmakarami@yahoo.com

Address: Department of Pharmacology, Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Received: 2021/03/10
Revised: 2021/05/03
Accepted: 2021/05/22

DOI: 10.29252/mlj.17.1.35

ABSTRACT

Background and objectives: Gentamicin is an aminoglycoside antibiotic used in the treatment of Gram-negative bacterial infections. One of the side effects of this antibiotic is nephrotoxicity. In this study, the protective effect of *Melissa officinalis L.* extract on diabetes- and gentamicin-induced nephrotoxicity was studied.

Methods: Forty male Wistar rats were randomly divided into four groups. The first group received distilled water, and the second group received *M. officinalis L.* extract (100 mg/kg) for 28 days. The third group received streptozocin (60 mg/kg) for 18 days, and then received gentamicin (80 mg/kg) on day 20 for 8 consecutive days. The fourth group received streptozocin, gentamicin, and *M. officinalis L.* extract for 28 days. Serum levels of blood urea nitrogen (BUN), creatinine, glucose, and amylases were measured. The right kidney was maintained in 10% formalin for hematoxylin and eosin staining, and oxidative stress markers in the left kidney were assessed.

Results: In the third group, serum BUN, creatinine, glucose, amylase, and malondialdehyde (MDA) increased, while glutathione peroxidase, superoxide dismutase, and catalase activities decreased significantly compared to the other groups ($P<0.05$). The extract of *M. officinalis L.* significantly inhibited the enhancement of serum creatinine, BUN, glucose, amylase, and MDA ($P<0.05$). Histological examinations showed that diabetes and gentamicin could lead to kidney damage by inducing necrosis and inflammation. Finally, the extract *of M. officinalis L.* could significantly reduce the adverse effects of both gentamicin and diabetes ($P<0.05$).

Conclusion: The extract of *M. officinalis L.* improves biochemical parameters and histological lesions in diabetic rats treated with gentamicin.

Keywords: Gentamicin, Streptozocin, Melissa, Kidney disease, Rats.

INTRODUCTION

Approximately five percent of the world's population has diabetes mellitus. Kidney disease is a common complication of diabetes, which can result in end-stage renal disease. The pathogenesis of diabetic nephropathy is diverse and may include abnormal glucose metabolism, hemodynamic alterations, oxidative stress, and some genetic factors. Diabetic nephropathy is characterized by glomerular hypertrophy, increased glomerular filtration rate, thickening of the basal membrane, and the aggregation of the extracellular matrix. Increased NAD(P)H oxidase activity, disruption of the mitochondrial respiratory chain, and increased polyol pathway flux elevate the production of reactive oxygen species in the kidneys of diabetic patients (1). Some clinical studies have shown that acute hyperglycemia could induce inflammation and increase apoptosis of kidney inherent cells (2). Gentamicin is an aminoglycoside antibiotic, which is widely used against Gram-negative bacterial infections in humans and animals. This antibiotic is known to cause acute renal failure in about 10-30% of patients, which is characterized by a rise in plasma creatinine, urea concentration, and renal tubular necrosis (3). In diabetic patients, susceptibility to infections increases because of the reduction of T-cell responses, neutrophil dysfunction, and humoral immunity impairment (4). Therefore, gentamicin may be used concurrently in diabetic patients for the treatment of Gram-negative bacterial infections, which further increases nephrotoxicity. *Melissa officinalis L.* is a medicinal herb belonging to the *Lamiaceae* family. This plant has been often used in traditional medicine for the treatment of some diseases such as depression, heart failure, bronchitis, colic, and hyperthyroidism. Furthermore, the antioxidant, anti-inflammatory, hepatoprotective, antiviral, antilipidemic of *M. officinalis L.* extracts have been demonstrated (5). Given these properties and the side effects of synthetic drugs, the present study aimed to evaluate effects of *M. officinalis L.* on gentamicin-induced renal failure in a diabetic rat model.

MATERIALS AND METHODS

Streptozocin (STZ) was purchased from Sigma, USA. Gentamicin was purchased from Zahravi Pharmaceutical Co., Iran. The plant of *M. officinalis L.* was obtained from Kashan (Iran) and then identified at the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, Tabriz University of Medical Sciences, Iran. The leaves (50 g) of the plant were homogenized in distilled water (1:5w/v). The extract was filtered with the aid of a vacuum pump and then centrifuged at 15 ℃ for 15 minutes at 5,000 rpm. The supernatant was stored at -20 ℃.

Forty, male Wistar rats weighing 220-250g were used in this study. All animals had access to food and water ad libitum. The animals were held in separate cages (4 per cage), at 23-25 ˚C, and under a 12-hour light-dark cycle. They become familiarized with the laboratory environment for one week. All experiments on the animals were carried out according to the guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication. No85-23, revised1996). The study protocol received approval from the local ethics committee (Ethical code: IR. Iaushab.19510501941002).

The rats were randomly divided into four groups. The first group (control) received 1 ml of distilled water by gavage for 28 days. The second group received the *M. officinalis L.* extract (100 mg/kg) by gavage for 28 days (5). The third group first received an intraperitoneal (i.p) injection of STZ (60 mg/kg) that was freshly dissolved in 0.1 M citrate buffer (pH =4.6) (6) and then 80 mg/kg gentamicin 20 days after the administration of STZ for 8 consecutive days (3). The fourth group received the *M. officinalis L.* extract, STZ, and gentamicin with the same dose and schedule as the second and third groups. Three days after the administration of STZ in the third and fourth groups, glucose concentration was measured with a glucometer (Beurer, Germany) using tail vein blood, and a blood glucose concentration of ≥ 250 mg/dl confirmed induction of diabetes.

The animals were anesthetized by ether 24 hours after the last drug administration and then sacrificed by cervical dislocation. Blood samples were taken from the heart. One of the kidneys was cut for histopathological studies. The other kidney was washed with saline and homogenate was prepared in 1.15% KCl solution. Then, the homogenates were centrifuged and the supernatant was used for

analysis. Blood samples were centrifuged at 3,000 rpm for 10 minutes at 4 °C. Serum creatinine and urea concentrations were determined by using commercial kits (Pars Azmun, Iran).

Serum amylase activity was also determined using a commercial kit (Pars Azmun, Iran) in the Alcyon300 autoanalyzer (Chema Diagnostica Crop. Monsano, Italy). Insulin level was measured with a rat insulin enzyme-linked immunosorbent assay kit (BT LAB, China).

Malondialdehyde (MDA) levels were determined using the thiobarbituric acid reactive substances method. The concentration of glutathione peroxidase (GPx) was determined according to Paglia and Valentine using the Randox Kit (United Kingdom). Superoxide dismutase (SOD) activity was assessed using the xanthine oxidase system (Randox, Ransod, United Kingdom). Catalase (CAT) activity was determined according to the method described by Aebi (7).

Kidney tissues were fixed in 10% formalin and phosphate buffer (pH 7.0) at room temperature for 48 hours. The tissues were embedded in paraffin. Microtome slices (5 µm thick) were prepared from the kidney tissue samples and then stained with hematoxylin-eosin. The stained samples were studied under a light microscope to investigate the details of renal structure in each group. All microscopic investigations were done by a pathologist who was blind to the study groups. A score of 0 to 3 was given to each tubular form based on the following criteria: score 0: no damage; score 1: tubular cell swelling, inflammation (presence of mononuclear cells), and necrosis in <25% of tubular cells; score 2: tubular cell swelling, inflammation, and necrosis in 25-50% of tubular cells; score 3: tubular cell swelling, inflammation, and necrosis in 50-75% of tubular cells (8).

Data were expressed as mean ± standard deviation. Intergroup differences were assessed using one-way ANOVA and Tukey's test for biochemical parameters. Nonparametric tests including the Kruskal-Wallis and Mann-Whitney U test were used for histological studies. All analyses were carried out in SPSS software (version 24), and P-values less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the levels of urea, creatinine, glucose, insulin, and amylase in the serum of rats in different study groups. The diabetes + gentamicin group had significantly higher blood urea nitrogen (BUN), creatinine, glucose, and amylase levels and significantly lower insulin level compared with other groups. These effects were reversed to normal in the rats that received the *M. officinalis L.* extract. On the other hand, there was no significant difference between the diabetes + gentamicin + *M. officinalis L.* group and the control and *M. officinalis L.* groups.

Table 1- The effect of *M. officinalis L.* extract on serum levels of blood urea nitrogen, creatinine, glucose, insulin, and amylase in different experimental groups (n= 10/group).

Groups	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Insulin (mlU/L)	Amylase (U/L)
Control	43.78 ± 7.4	0.51 ± 0.11	126 ± 36	10.7 ± 0.73	70 ± 17
M. officinalis L.	44.6 ± 8.1	0.58 ± 0.51	132 ± 29.5	11.01± 0.61	68.4 ± 12
Diabetes+gentamicin	89 ± 9.6[a]	1.08 ± 0.27[a]	280 ± 13.3[a]	8.5 ± 1.1[a]	557.3 ± 79[a]
Diabetes+gentamicin+*M. officinalis L.*	52.5 ± 14.77	0.64 ± 0.91	148 ± 27.7	11.41± 1	69.6 ±26

Data are presented as mean ± standard deviation for 10 rats in each group. [a] statistically significant difference compared with the control group (P≤ 0.05).

Moreover, MDA levels in the kidney tissues were significantly higher in the diabetes + gentamicin group compared with the control and *M. officinalis L.* groups. In the diabetes + gentamicin + *M. officinalis L.* group, the mean MDA level was significantly lower than that in the diabetes + gentamicin group; however, this difference was not significantly significant compared with the control and *M. officinalis L.* groups (Table 2). The results also showed that GPX, SOD, and catalase levels in the rats in the diabetes + gentamicin group were significantly lower compared with the control and the *M. officinalis L.* groups. In the diabetes + gentamicin + *M. officinalis L.* group, the mean GPX, SOD, and CAT levels were significantly increased compared with the diabetes + gentamicin group; however, no significant difference was observed between the diabetes + gentamicin + *M. officinalis L.* group and the control and *M. officinalis L.* groups.

Table 2.- The effects of *M. officinalis L.* on MDA, GPX, SOD, and CAT levels in the kidney tissue samples of each group (n= 10/group).

Groups	MDA (nmol/mg protein)	GPX (U/mg protein)	SOD (U/mg protein)	Catalase (K/mg protein)
Control	0.074 ±.0046	26.5 ± 2.5	7.1 ± 0.55	13.8 ±2.2
M. officinalis L.	0.074 ± 0.003	27.7 ± 1.9	6.7 ± 0.67	14.7 ± 1.9
Diabetes + gentamicin	0.085 ± 0.015 [a]	21 ± 1.6 [a]	6.01 ± 0.52 [a]	7.6 ± 1.5 [a]
Diabetes + gentamicin + *M. officinalis L.*	0.075 ± .002	25.9 ± 1.7	6.6 ± 0.5	12.1 ± 2.3

Data are presented as mean ± standard deviation for 10 rats in each group. [a] statistically significant difference compared with the control group (P≤ 0.05).

Histological examination of the kidney tissue from the control and *M. officinalis L.* groups was normal (Figure 1, 2).

Histological scores of hyaline casts, tubular necrosis, tubular cell swelling, and inflammation increased significantly in the samples from the diabetes + gentamicin group compared with other groups (Figure 3).

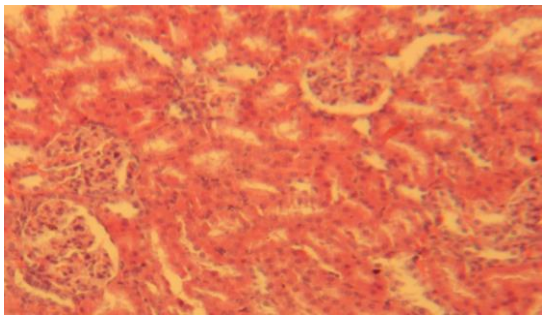

Figure 1-Histological image of kidney tissue section obtained from rats in the control group showing normal renal histoarchitecture (hematoxylin-eosin staining, ×10 magnification).

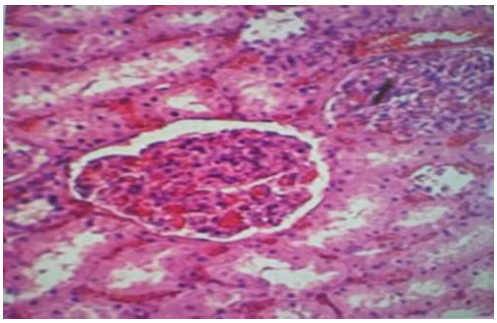

Figure 2- Histological image of kidney tissue section obtained from rats in the *M. officinalis L.* group showing normal renal histoarchitecture (hematoxylin-eosin staining, ×10 magnification).

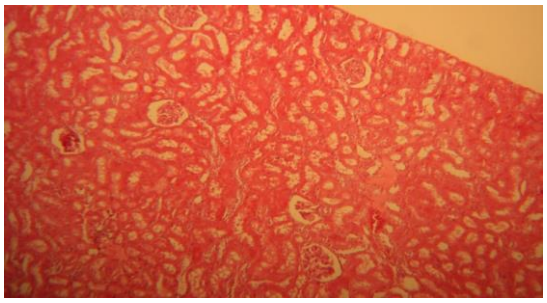

Figure 3- Histological image of kidney tissue section obtained from rats in the diabetes + gentamicin group showing hemorrhagic tubular necrosis, tubular cell swelling, and inflammation (hematoxylin-eosin staining, ×10 magnification).

Treatment with *M. officinalis L.* preserved the normal morphology of the kidney (Figure 4).

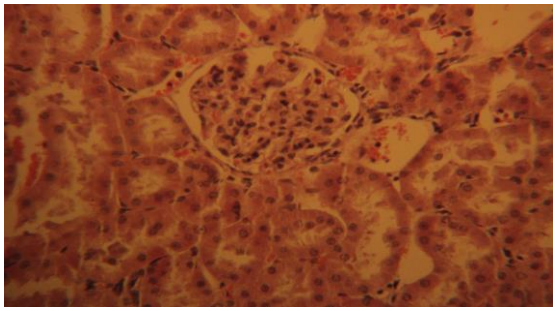

Figure 4- Histological image of kidney tissue section obtained from rats in the diabetes + gentamicin + *M. officinalis L.* group showing a near normal morphology

DISCUSSION

Diabetic nephropathy is one of the most common complications of diabetes (9). Gentamicin can also induce acute tubular necrosis and renal function impairment. Our findings showed that induction of diabetes with STZ as well as gentamicin administration (80 mg/kg BW) result in nephrotoxicity, with manifestations such as reduction of glomerular filtration rate and increase in the levels of serum creatinine and BUN. Diabetes and gentamicin both increased serum levels of amylase and decreased serum levels of insulin. Glomerular damage was associated with a decrease in GPX, SOD, and CAT levels as well as an increase in MDA levels in the kidney tissues, which indicated lipid peroxidation. Treatment with the *M. officinalis L.* extract reversed these parameters to near-normal levels. Erdem et al. (10) and Cohen et al. (11) stated rats that the administration of gentamicin significantly increased BUN and serum creatinine levels in rats. Murali et al. explained that rats receiving STZ showed a significant increase in blood glucose, creatinine, and BUN levels (12). Our results are in agreement with the findings of the mentioned studies when gentamicin and STZ are used simultaneously. In studies by Zhang et al. (13) and Ghanbari et al. (6), CAT, SOD, and GSH activity reduced, whereas MDA levels increased in STZ-induced diabetic rats compared to control rats. In a study by Yazar et al., the administration of aminoglycoside antibiotics to rats significantly decreased renal GSH levels but did not alter SOD, GPX, and MDA levels significantly (14).

Oxidative stress is the result of an imbalance between oxidants and antioxidants. The aggregated reactive oxygen species could react with polyunsaturated fatty acids and generate lipid peroxidation in the kidney tissues, which results in kidney damage or toxicity. The extracts of *M. officinalis L.* have protective antioxidant effects on several tissues. Some studies showed that gentamicin administration in rats elevates the production of H_2O_2 in the kidneys, thereby increasing the production of superoxide anions and hydroxyl radicals. Safaeian et al. showed that *M. officinalis L.* has antioxidant and cytoprotective effects against H_2O_2-induced oxidative stress in human umbilical vein endothelial cells (15). Moreover, Shin et al. demonstrated that the destruction of pancreatic islets could be prevented by *M. officinalis L.* in diabetic rats (16). In another study, the treatment of rats with the *M. officinalis L.* extract had promising effects in terms of preventing damage to pancreatic islets and beta cells (17). In a study by Seif et al., treatment of animals with malathion alone caused perivascular edema associated with inflammatory cells infiltration in the kidneys, hyperplasia of intrahepatic bile ducts, and focal hepatic necrosis, which were all improved by administration of *M. officinalis L.* (18). These results are in line with our histological findings. The histopathological findings of this study indicated the protective role of *M. officinalis L.* against gentamicin-induced renal failure in diabetic rats.

CONCLUSION

In the present study, the administration of gentamicin caused histopathological and biochemical alterations in the kidney tissues of rats; however, these alterations could be reversed to normal using the *M. officinalis L.* extract. The extract of *M. officinalis L.* could reverse the negative effects of both gentamicin

and diabetes mellitus simultaneously, possibly by inhibiting the free radical-mediated processes. However, the exact mechanisms through which *M. officinalis L.* could exert such effects remain to be explained by future studies.

ACKNOWLEDGMENTS

The authors acknowledge the technical support of the Drug Applied Research Center of Tabriz University of Medical Sciences.

DECLARATIONS

Funding

This work was part of A.R Nasiri̓s DVM project, which was supported by the Faculty of Veterinary Medicine, Islamic Azad University, Shabestar Branch.

Ethics approvals and consent to participate

All experiments on the animals were carried out according to the guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication. No85-23, revised1996). The study protocol was approved by the local ethics committee (Ethical code: IR. Iaushab.19510501941002).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. Mi N, Zhang XJ, Ding Y, Li GH, Wang WD, Xian HX, et al. *Branched-chain amino acids attenuate early kidney injury in diabetic rats*. Biochem Biophys Res Commun. 2015; 466(2): 240-6 [View at Publisher] [DOI:10.1016/j.bbrc.2015.09.017] [PubMed] [Google Scholar]

2. Wang JY, Yang JH, Xu J, Jia JY, Zhang XR, Yue XD, et al. *Renal tubular damage may contribute more to acute hyperglycemia induced kidney injury in non-diabetic conscious rats.* J Diabetes Complications. 2015; 29(5):621-8. [View at Publisher] [DOI:10.1016/j.jdiacomp.2015.04.014] [PubMed] [Google Scholar]

3. Al-Majed AA, Mostafa AM, Al-Rikabi AC, Al-Shabanah OA. *Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats.* Pharmacol Res. 2002 ;46(5):445-51. [View at Publisher] [DOI:10.1016/S1043661802001251] [PubMed] [Google Scholar]

4. Casqueiro J, Casqueiro J, Alves C. *Infections in patients with diabetes mellitus: A review of pathogenesis.* Indian J Endocrinol Metab. 2012 ;16 Suppl 1(Suppl1): S27-36. [DOI:10.4103/2230-8210.94253] [PubMed] [Google Scholar]

5. Martins EN, Pessano NT, Leal L, Roos DH, Folmer V, Puntel GO, et al. *Protective effect of Melissa officinalis aqueous extract against Mn-induced oxidative stress in chronically exposed mice*. Brain Res Bull. 2012; 87(1): 74-9. [View at Publisher] [DOI:10.1016/j.brainresbull.2011.10.003] [PubMed] [Google Scholar]

6. Ghanbari E, Nejati V, Azadbakht M. *Protective effect of royal jelly against renal damage in streptozotocin induced diabetic rats.* Iranian Journal of Toxicology. 2015; 28(9): 1259-1263. [Google Scholar]

7. Aebi H. *Catalase in vitro.* Methods in Enzymology. 1984;105: 121-126 . [View at Publisher] [DOI:10.1016/S0076-6879(84)05016-3] [PubMed] [Google Scholar]

8. Hur E, Garip A, Camyar A, Ilgun S, Ozisik M, Tuna S, et al. *The effects of vitamin d on gentamicin-induced acute kidney injury in experimental rat model*. Int J Endocrinol. 2013;2013:313528. [DOI:10.1155/2013/313528] [PubMed] [Google Scholar]

9. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J,et al. *Diabetic kidney disease: a report from an ADA Consensus Conference*. Am J Kidney Dis. 2014 ;64(4):510-33. [View at Publisher] [DOI:10.1053/j.ajkd.2014.08.001] [PubMed] [Google Scholar]

10. Erdem A, Gündoğan NU, Usubütün A, Kilinç K, Erdem SR, Kara A,et al. *The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats.* Nephrol Dial Transplant. 2000 ;15(8):1175-82 [View at Publisher] [DOI:10.1093/ndt/15.8.1175] [PubMed] [Google Scholar]

11. Cohen L, Lapkin R, Kaloyanides GJ. *Effect of gentamicin on renal function in the rat*. J Pharmacol Exp Ther. 1975 ;193(1):264-73. [PubMed] [Google Scholar]

12. Murali B, Goyal R. *Effect of chronic treatment with losartan on streptozotocin induced diabetic nephropathy*. Clinical and Experimental Hypertension. 2001;23(7):513-20. [View at Publisher] [DOI:10.1081/CEH-100106822] [PubMed] [Google Scholar]

13. 1081/CEH-100106822 [DOI:10.1081/CEH-100106822] [PubMed] [Google Scholar]

14. Zhang S, Xu H, Yu X, Wang Y, Sun F, Sui D. *Simvastatin ameliorates low-dose streptozotocin-induced type 2 diabetic nephropathy in an experimental rat model*. Int J Clin Exp Med. 2015 15;8(4):6388-96. [PubMed] [Google Scholar]

15. Yazar E, Elmas M, Altunok V, Sivrikaya A, Oztekin E, Birdane YO. *Effects of aminoglycoside antibiotics on renal antioxidants, malondialdehyde levels, and some serum biochemical parameters*. Canadian journal of veterinary research. 2003;67(3): 239-240. [PubMed] [Google Scholar]

16. Safaeian L, Sajjadi SE, Javanmard SH, Montazeri H, Samani F. *Protective effect of Melissa officinalis extract against H2O2-induced oxidative stress in human vascular endothelial cells. Res* Pharm Sci. 2016 ;11(5):383-389. [DOI:10.4103/1735-5362.192488] [PubMed] [Google Scholar]

17. Shin Y, Lee D, Ahn J, Lee M, Shin SS, Yoon M. *The herbal extract ALS-L1023 from Melissa officinalis reduces weight gain, elevated glucose levels and β-cell loss in Otsuka Long-Evans Tokushima fatty rats*. J Ethnopharmacol. 2021 10;264:113360. [View at Publisher] [DOI:10.1016/j.jep.2020.113360] [PubMed] [Google Scholar]

18. Ashkani-Esfahani S, Ebrahimi A, Bahmani-Jahromi M, Nadimi E, Arabzadeh H, Jalalpour MH, et al. *The Effect of Melissa officinalis Extract on Streptozotocin-Induced Diabetes in Rats: A Stereological Study on Pancreatic Islets and Beta-cells.* Journal of Advances in Medical and Biomedical Research. 2021;29(132):34-40. [DOI:10.30699/jambs.29.132.34] [Google Scholar]

19. Sief M, Khalil F, Abou-Arab A, Abou Donia M, El-Sherbiny A, Mohamed S. *Ameliorative role of Melissa officinalis against hepatorenal toxicities of organophosphorus malathion in male rats.* MedCrave Online Journal of Toxicology. 2015;1(3):00013. [DOI:10.15406/mojt.2015.01.00014] [Google Scholar]

How to Cite:
Nasiri AR, Karamibonari AR[Protective Effects of *Melissa officinalis* L. Extract on Gentamicin-induced Renal Failure in Diabetic Rats]. mljgoums. 2023; 17(1):35-41 DOI: 10.29252/mlj.17.1.