Ulus Cerrahi Derg 2016; 32: 233-237
DOI: 10.5152/UCD.2015.3224

The effects of melatonin on liver functions in arsenic-induced liver damage

İlhan Bali[1], Bülent Bilir[2], Seyfi Emir[1], Filiz Turan[3], Ahsen Yılmaz[4], Tuba Gökkuş[4], Murat Aydın[4]

ABSTRACT

Objective: Arsenic exposure is increasing in communities due to environmental pollution and industrial development. Arsenic is toxic to organ systems because it causes oxidative stress, enzymatic inhibition, and damage to protein structures. The liver, for example, is an organ that may be damaged by arsenic, and this damage may cause various clinical conditions like hepatic failure or cancer. Melatonin is a hormone that acts like an antioxidant, an anti-inflammatory agent, and a cytoprotective agent. In this study, we aimed to evaluate melatonin's protective effects on livers damaged by arsenic toxicity.

Materials and Methods: Twenty-four Sprague-Dawley male rats were classified into three groups: a control group, an arsenic applied group, and an arsenic plus 10 mg/kg melatonin applied group. At the end of the fifteen-day experiment, the rats were sacrificed. Albumin, interleukin-6 (IL-6), total protein, alanine transaminase, aspartate transaminase, macrophage migration inhibitory factor, and monocyte chemotactic protein-1 measurements were obtained.

Results: In rats with liver damage due to arsenic exposure, melatonin administration significantly decreased the levels of IL-6, macrophage migration inhibitory factor, and monocyte chemotactic protein-1 ($p<0.001$, $p=0.02$ and $p=0.04$, respectively).

Conclusion: After evaluating liver enzymes and inflammatory markers, this study determined that melatonin exposure improves liver tissue damage caused by arsenic exposure, with the degree of improvement varying based on the levels of arsenic exposure.

Keywords: Arsenic, interleukin-6, macrophage migration inhibitory factor, melatonin, monocyte chemotactic protein 1

INTRODUCTION

Arsenic, one of the most prevalent element on earth, belongs to the group of heavy metals (1, 2). Inorganic arsenic, which is the most prevalent type, dominates sea water, surface waters, and underground water. Conversely, organic forms of arsenic are found within natural gas and petroleum (3). Arsenic can easily change oxidation steps and chemical forms in nature. Arsenic valence and type are affected by redox potential, pH of the water, microbiologic activity, and the presence of ions like sulfur, iron, and calcium (3). In recent years, arsenic exposure has increased because of pollution and industrial development (4). Particularly, the agriculture chemicals used in dyes, ceramics, cancer drugs, mining operations, and herbicides and insecticides are leading routes of exposition (5-7). Long periods of arsenic intake by mouth or through inhalation cause both metabolic and structural changes in the hepatocyte mitochondria (8). Arsenic causes hepatocyte degeneration, inflammation, and necrosis. It also causes increased apoptosis, oxidative damage, and damage at lipid peroxidation (9, 10).

Melatonin is primarily secreted from a neuroendocrine organ called the pineal gland. It is also secreted from the skin, the retina, testicles, bone marrow, thrombocytes, lymphocytes, and the gastrointestinal system (11-17). Melatonin synthesis and release are both stimulated by darkness and repressed by light. Melatonin is a strong free radical sweeper agent, as demonstrated in both in vivo and in vitro studies (18-20). It is much more powerful than all known antioxidants due to its free radical catching effects. Studies have shown that it is a more potent antioxidant than vitamin E and glutathione (21, 22). This is primarily because melatonin can dissolve in either water or fat, thus affecting all cell components (19-23). Melatonin also reportedly has regulatory effects on immunity and anti-inflammation (24-26). The aim of this study was to investigate the regulatory effects of melatonin on arsenic toxicity by monitoring hepatic enzymes and inflammatory markers in rats whose livers were intentionally damaged by arsenic for experimental purposes.

MATERIAL AND METHODS

Study Group

Twenty-four male Sprague-Dawley rats were used in the experiment. Rats were kept at 22°C under illumination control (14 hours light/10 hours dark cycle). They were monitored in standard cages in an

[1]Department of General Surgery, Namık Kemal University School of Medicine, Tekirdağ, Turkey
[2]Department of Internal Medicine, Namık Kemal University School of Medicine, Tekirdağ, Turkey
[3]Department of Anesthesiology and Reanimation, Namık Kemal University School of Medicine, Tekirdağ, Turkey
[4]Department of Medical Biochemistry, Namık Kemal University School of Medicine, Tekirdağ, Turkey

Address for Correspondence
İlhan Bali
e-mail: ilhanbali@yahoo.com

Received: 08.06.2015
Accepted: 04.08.2015
Available Online Date : 27.10.2016

Available online at
www.ulusalcerrahidergisi.org

air-conditioned room. All rats weighed 250-300 g and were obtained from the animals laboratory at Namık Kemal University. The use of laboratory animals, animal experiments, and procedures were performed in accordance with national guidelines for care and were approved by the Namık Kemal University Animal Experiment Local Ethics Committee (NKU-HADYEK Decision No: 03/2015). The group formation was planned as follows:

The control group (n=8) was given 10 mL/kg of 0.9% NaCl every day through intragastric gavage for 15 days.

The arsenic group (n=8) was given 10 mg/kg of sodium arsenite (Sodium metaarsenite, Sigma-Aldrich Sodium metaarsenite >=90% Steinheim, Germany) every day by dissolving it into distilled water through intragastric gavage for 15 days.

The arsenic and melatonin groups (n=8) were given 10 mg/kg of dissolved sodium arsenite and 10 mg/kg of dissolved melatonin (Melatonin, Sigma-Aldrich Melatonin, Solid Steinheim, Germany) in 10% ethyl alcohol through intragastric gavage every day for 15 days.

At the end of the 15-day experiment period, all rat weights were measured, and they were then sacrificed. Blood was drawn from the left ventricle under deep Ketamine (90 mg/kg) and Xylazine (10 mg/kg) anesthesia. For chemical measurements, samples were drawn into gelled vacuum tubes and then centrifuged at 2750 g RCF for 10 minutes in a cool centrifuge. Serums were kept at -80°C until the measurements were taken.

Chemical Measurements

All chemical measurements were performed using the Cobas e6000 (e501, Roche Diagnostics) system with commercial analyses kits. Serum macrophage Migration Inhibitor Factor (MIF) levels were also measured (Rat MIF ELISA; Cusabio Biotech Co. Ltd.). The MCP-1 level was measured using ELISA kits (Rat MCP-1 ELISA; Cusabio Biotech Co. Ltd.). The MIF and MCP-1 minimum measurement levels were 1.6 pg/mL and 1.7 pg/mL, respectively. The ELISA kit (Rat IL-6 Platinum ELISA eBioscience Bender MedSystems GmbH) was used to determine serum IL-6 levels, and the results were expressed in pg/mL.

Statistical Analysis

Statistical Package for the Social Sciences 16.0 software for Windows (SPSS Inc.; Chicago, IL, USA) was used to statistically evaluate the data. A Shapiro-Wilk test was used for group distribution (i.e., homogeneity), a Student's t-test (i.e., for the homogenous group and parametric values), and a Mann-Whitney U test (i.e., for the heterogeneous group and non-parametric values) was used for binary comprehension. A p value <0.05 was accepted as statistically significant. Numerical values were expressed as average ± standard deviation (SD) or median and minimum-maximum values.

RESULTS

When the arsenic group and the control group were compared, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were higher in the arsenic group ($p<0.001$ and $p<0.001$, respectively). Significant decreases were observed in AST and ALT levels in the melatonin group when compared with the arsenic group ($p<0.002$ and $p<0.001$, respectively). There was a significant difference ($p=0.029$) in the albumin level between arsenic and the control groups, while no significant differences were found between the melatonin and the arsenic groups. When the arsenic group and the control group were compared according to Il-6, MIF and monocyte chemotactic protein 1 (MCP-1), the levels were found to be significantly higher in the arsenic group ($p<0.001$, $p<0.001$, and $p<0.001$, respectively). Significant decreases at these levels were found in the melatonin group when compared with the arsenic group ($p<0.001$, $p<0.02$, and $p=0.04$ respectively). All measurement parameters were presented in detail in Figure 1 and Table 1.

DISCUSSION

Oxidative stress plays an important role in liver damage that has been caused by arsenic toxicity. Oxidative stress is defined as imbalances between antioxidant defense mechanisms and the production of free radicals, which cause peroxidation of cell lipid layers. Intracellular and extracellular lipid peroxidation increases cell, tissue, and organ damage. Also, arsenic may inhibit many enzymes and damage protein structures. The cytokines secreted after cellular damage increases the migration of inflammation to cells and tissues, so the inflammatory period is triggered (27, 28).

The hormone melatonin plays a role in physiological functions like regulating the endocrine system, increasing immune functions, regulating smooth muscle tonus, and repressing gonadal functions (29). It also reportedly regulates interleukin responses, saves cellular structures in the liver, and increases survival through the antioxidant effect (30).

Many researchers reported that melatonin's antioxidant effects can repair liver damage caused by heavy metals and accumulations of toxic materials (30-32). However, its anti-inflammatory effects have not yet been proven. In this study, hepatic enzymes, IL-6, MIF, and MCP-1 were used to evaluate the protective effects of melatonin in livers damaged by arsenic.

Table 1. Results of biochemical measurements at study and control groups

Group	Control	Arsenic	Melatonin
ALT (IU/L)	18.2±2.4	29.4±3.2[a]	20.6±1.5[c]
AST (IU/L)	109.1±12.9	150.1±22.5[a]	116.1±11.4[d]
Albumin (g/dL)	3.9±0.2	3.7±0.1[b]	3.7±0.2
Total Protein (g/dL)	5.5±0.3	5.7±0.4	5.6±0.3
IL-6 (pg/mL)	12.84 (10.5-13.9)	26.7 (20.1-30.7)[a]	14.9 (13.8-17.1)[c]
MCP-1 (pg/mL)	5.3±0.7	6.7±0.6[a]	5.4±1.0[d]
MIF (pg/mL)	50.7±6.5	74.4±10.8[a]	57.7±6.3[d]

AST: aspartate aminotransferase; ALT: alanine aminotransferase; IL-6: interleukin-6; MCP-1: monocyte chemotactic protein 1; MIF: macrophage migration inhibitor factor
[a]p<0.001 compared with control group. [b]p<0.05 compared with control group. [c]P<0.001 compared with arsenic group. [d]p<0.05 compared with arsenic group.

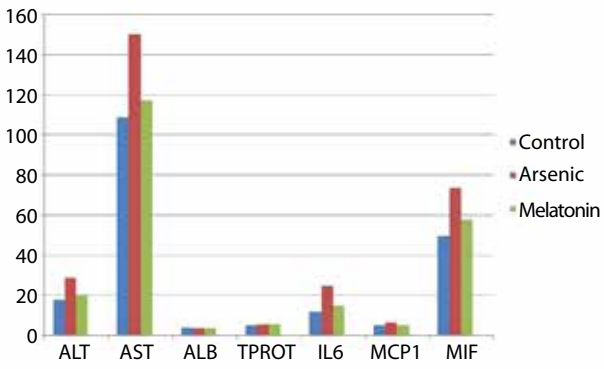

Figure 1. Biochemical measurement results of control, arsenic and melatonin groups
AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TPROT: total protein

Two enzymes in particular, ALT and AST, enable amino group transfer during amino acid metabolism and gluconeogenesis (33, 34). Increases in aminotransferase serum levels are important indicators for acute or chronic liver damage (35). Arsenic reportedly has toxic effects on the liver and chronic exposure can cause different clinical scenarios ranging from liver damage to cancer (36).

It was reported (37-39) that 10-100 mg/kg melatonin application significantly decreased AST and ALT levels that were elevated because of substances like carbon tetrachloride, dimethyl nitrosamine, and acetaminophen that had caused liver toxicity in rats. In our study, AST and ALT levels were higher in rats administered arsenic when compared with the control group. Additionally, applications of 10 mg/kg melatonin caused significant decreases in AST and ALT levels. These findings lead us to believe that melatonin has the effect of repairing liver damage caused by arsenic.

Interleukin-6 (IL-6) is secreted from monocytes, fibroblasts, endothelium cells, and B lymphocytes, but its main secretion source is T lymphocytes, and it basically activates monocytes and macrophages. It is a pro-inflammatory cytokine that stimulates B lymphocyte differentiation and antibody secretion (40). Although melatonin has anti-inflammatory effects, clinical studies about the effects of melatonin on IL-6 are sometimes contradictory. Srinivasan et al. (41) reported that melatonin increases IL-2, IL-6, IL-12, and interferon (IFN) gamma levels by stimulating cytokine production, and that it has an oncostatic effect. Conversely, Broncel et al. (42) reported that melatonin decreases IL-6, IL-12, TNF-alpha, and IFN gamma levels. In our study, IL-6 levels were significantly increased in the arsenic group when compared with the control group. There were significant decreases in the melatonin group when compared with the arsenic group. These findings support the conclusion that melatonin decreases cytokine secretion and exhibits a hepatoprotective effect via an anti-inflammatory mechanism.

Animal studies have determined that arsenic exposure stimulates IL-6, MCP-1, and vascular endothelial growing factor gene expression. It also triggers an inflammatory period where applied alpha lipoic acid decreases proinflammatory molecule levels (43). In another study, arsenic reportedly stimulated production of proinflammatory cytokines, IL-1B, IL-6, MCP-1, and C reactive protein through inducible nitric oxide synthase (iNOS) in the vascular system (27). There are no data in the literature on the effects of melatonin on MCP-1 levels during arsenic toxicity. In our study, MCP-1 levels were lowered with application of melatonin to liver tissues with arsenic toxicity. Also, proinflammatory cytokines were repressed in either the liver or damaged tissues. This lead to the conclusion that melatonin is an important factor for healing.

The MIF is a complex protein showing the properties of an inflammatory cytokine, a neuroendocrine hormone, and an enzyme, while its main effect is decreasing macrophage migration (44). Additionally, MIF exhibits proinflammatory effects by secreting NO and activating the cyclooxygenase pathway as well as by stimulating tumor necrosis factor-alpha, IL-1B, IL-2, IL-6, IL-8, IFN-c, and adhesion molecules (45). Furthermore, MIF neutralization is decreased. According to different animal study models (46, 47), its release increases liver damage caused by alcohol and various toxic substances. Getting MIF levels under control is important in the treatment of many inflammatory diseases. There have been clinical studies on MIF antagonists over the last few years (48). In our study, melatonin significantly decreased MIF levels that had been increased by arsenic exposure. Proinflammatory cytokine release was decreased to address inflammatory damage, and macrophage migration was resolved.

CONCLUSION

This study concerning liver enzymes and inflammatory markers detected that melatonin has therapeutic effects on liver tissues that were damaged by arsenic exposure.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Namık Kemal University.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - İ.B.; Design - İ.B., B.B., M.A.; Supervision - S.E., F.T.; Resources - İ.B., B.B., S.E.; Materials - M.A.; Data Collection and/or Processing - A.Y., T.G.; Analysis and/or Interpretation - İ.B., B.B., S.E., M.A.; Literature Search - İ.B., M.A., A.Y., F.T.; Writing Manuscript - İ.B., M.A.; Critical Review - S.E., B.B., F.T.; Other - A.Y., T.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Lombi E, Holm PE: Metalloids, soil chemistry and the environment. Adv Exp Med Biol 2010; 679: 33-44. [CrossRef]
2. Wang P, Sun G, Jia Y, Meharg AA, Zhu Y. A review on completing arsenic biogeochemical cycle: microbial volatilization of arsines in environment J Environ Sci 2014; 26: 371-381. [CrossRef]
3. U.S. EPA, Arsenic Treatment Technologies for Soil, Waste and Water, U.S. EPA/National Service Center for Environmental Publications, Cincinnati, 2002.

4. P Mangalgiri K, Adak A, Blaney L. Organoarsenicals in poultry litter: Detection, fate, and toxicity. Environ Int 2015; 75: 68-80. [CrossRef]
5. Mandal BK, Suzuki KT. Arsenic round the world: a review. Talanta 2002; 58: 201-235. [CrossRef]
6. Moore K. Treatment of arsenic contaminated groundwater using oxidation and membrane filtration, master of applied science in civil engineering, University of Waterloo, 2005.
7. Tchounwou PB, Wilson BA, Abdelghani AA, Ishaque AB, Patlolla A. Differential cytotoxicity and gene expression in human liver carcinoma (HepG2) cells exposed to arsenic trioxide and monosodium acid methanearsonate (MSMA). Int J Mol Sci 2002; 3: 1117-1132. [CrossRef]
8. Fowler BA, Woods JS, Schiller CM. Ultrastructural and biochemical effects of prolonged oral arsenic exposure on liver mitochondria of rats. Environ Health Perspect 1977; 19: 197-204. [CrossRef]
9. Bashir S, Sharma Y, Irshad M, Gupta SD, Dogra TD. Arsenic-induced cell death in liver and brain of experimental rats. Basic Clin Pharmacol Toxicol 2006; 98: 38-43. [CrossRef]
10. Bashir S, Sharma Y, Irshad M, Nag TC, Tiwari M, Kabra M, et al. Arsenic induced apoptosis in rat liver following repeated 60 days exposure. Toxicology 2006; 217: 63-70. [CrossRef]
11. Tosini G, Menaker M. The clock in the mouse retina: melatonin synthesis and photoreceptor degeneration. Brain Res 1998; 789: 221-228. [CrossRef]
12. Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestroni JM. Evidence for melatonin synthesis in Mouse and human bone marrow cells. J Pineal Res 2000; 28: 193-202. [CrossRef]
13. Slominski A, Tobin DJ, Zmijewski MA, Wortsman J, Paus R. Melatonin in the skin: synthesis, metabolism and functions. Trends Endocrinol Metab 2008; 19: 17-24. [CrossRef]
14. Champier J, Claustrat B, Besançon R, Eymin C, Killer C, Jouvet A, et al. Evidence for tryptophan hydroxylase and hydroxy-indol-O-methyl-transferase mRNAs in human blood platelets. Life Sci 1997; 60: 2191-2197. [CrossRef]
15. Carrillo-Vico A, Calvo JR, Abreu P, Lardone PJ, García-Mau ri-o S, Reiter RJ, et al. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. FASEB J 2004; 18: 537-539. [CrossRef]
16. Tijmes M, Pedraza R, Valladares L. Melatonin in the rat testis: evidence for local synthesis. Steroids 1996; 61: 65-68. [CrossRef]
17. Bubenik GA: Gastrointestinal melatonin: localization, function, and clinical relevance. Dig Dis Sci 2002; 47: 2336-2348. [CrossRef]
18. Reiter RJ, Tan DX. Melatonin: A novel protective agent against oxidative injury of the ischemic-reperfused heart. Cardiovasc Res 2003; 58: 10-19. [CrossRef]
19. Cruz MH, Leal CL, Cruz JF, Tan DX, Reiter RJ. Essential actions of melatonin in protecting the ovary from oxidative damage. Theriogenology 2014; 82: 925-932. [CrossRef]
20. Reiter DJ, Tan DX, Kim SJ, Wenbo QI. Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. Proc West Pharmacol Soc 1998; 41: 229-236.
21. Reiter R, Garcia JJ, Tang L, Munoz-Hoyos A. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 1997; 60: 2255-2257. [CrossRef]
22. Çağlıkülekçi M, Bilgin Ö, Canbaz H, Dirlik M, Bağdatoğlu Ö, Üstünsoy B, ve ark. Deneysel hepatik iskemi reperfüzyon modelinde melatonin uygulamasının kan ve karaciğer doku lipid peroksidasyonuna etkisi. Ulus Cerrahi Derg 2006; 22: 93-98.
23. Costa EJ, Shida CS, Biaggi MH, Ito AS, Lamy-Freund MT. How melatonin interacts with lipid bilayers: A study by fluorescence and ESR spectroscopies. F.E.B.S. Lett 1997; 416: 103-106. [CrossRef]
24. Cuesta S, Kireev R, Forman K, García C, Escames G, Ariznavarreta C, et al. Experimental Gerontology Melatonin improves inflammation processes in liver of senescence-accelerated prone male mice (SAMP8) Exp Gerontol 2010; 45: 950-956. [CrossRef]
25. Shin IS, Park JW, Shin NR, Jeon CM, Kwon OK, Kim JS, et al. Melatonin reduces airway inflammation in ovalbumin-induced asthma. Immunobiology 2014; 219: 901-908. [CrossRef]
26. Da Rosa DP, Forgiarini LF, e Silva MB, Fiori CZ, Andrade CF, Martinez D, et al. Antioxidants inhibit the inflammatory and apoptotic processes in an intermittent hypoxia model of sleep apnea. Inflamm Res 2015; 64: 21-29. [CrossRef]
27. Wang L, Kou MC, Weng CY, Hu LW, Wang YJ, Wu MJ. Arsenic modulates heme oxygenase-1, interleukin-6, and vascular endothelial growth factor expression in endothelial cells: roles of ROS, NF-κB, and MAPK pathways. Arch Toxicol 2012; 86: 879-896. [CrossRef]
28. Sumedha N, Miltonprabu S. Diallyl trisulfide ameliorates arsenic-induced hepatotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in rats. Hum Exp Toxicol 2014; pii: 0960327114543933.
29. Brzezinski A. Melatonin in humans. N Engl J Med 1997; 336: 186-195. [CrossRef]
30. Pal S, Chatterjee AK. Possible beneficial effects of melatonin supplementation on arsenic-induced oxidative stress in Wistar rats. Drug Chem Toxicol 2006; 29: 423-433. [CrossRef]
31. Sigala F, Theocharis S, Sigalas K, Markantonis-Kyroudis S, Papalabros E, Triantafyllou A, et al. Therapeutic value of melatonin in an experimental model of liver injury and regeneration. J Pineal Res 2006; 40: 270-279. [CrossRef]
32. Ohta Y, Kongo M, Sasaki E, Nishida K, Ishiguro I. Therapeutic effect of melatonin on carbon tetrachloride-induced acute liver injury in rats. J Pineal Res 2000; 28: 119-126. [CrossRef]
33. Wroblewski F, Ladue JS. Serum glutamic pyruvic transaminase in cardiac with hepatic disease. Proc Soc Exp Biol Med 1956; 91: 569-571. [CrossRef]
34. Sette LH, Almeida Lopes EP. Liver enzymes serum levels in patients with chronic kidney disease on hemodialysis: a comprehensive review. Clinics 2014; 69: 271-278. [CrossRef]
35. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ 2005; 172: 367-379. [CrossRef]
36. Liu J, Waalkes MP. Liver is a target of arsenic carcinogenesis. Toxicol Sci 2008; 105: 24-32. [CrossRef]
37. Kus I, Ogeturk M, Oner H, Sahin S, Yekeler H, Sarsilmaz M. Protective effects of melatonin against carbon tetrachlorideinduced hepatotoxicity in rats: a light microscopic and biochemical study. Cell Biochem Funct 2005; 23: 169-174. [CrossRef]
38. Jung KH, Hong SW, Zheng HM, Lee DH, Hong SS. Melatonin downregulates nuclear erythroid 2-related factor 2 and nuclear factor-kappaB during prevention of oxidative liver injury in a dimethylnitrosamine model. J Pineal Res 2009; 47: 173-183. [CrossRef]
39. Sener G, Sehirli AO, Ayanoğlu-Dülger G. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. J Pineal Res 2003; 35: 61-68. [CrossRef]
40. Karadağ R, Koca C, Totan Y, Yağcı R, Aydin M, Karadağ AS, et al. Comparison Of Serum Levels Of Il-6, Il-8, Tnf-A, C Reactive Protein And Heat Shock Protein 70 İn Patients With Active or İnactive Behçet's Disease. Turk J Med Sci 2010; 40: 57-62.
41. Srinivasan V, Spence DW, Pandi-Perumal SR, Trakht I, Cardinali DP. Therapeutic actions of melatonin in cancer: possible mechanisms. Integr Cancer Ther 2008; 7: 189-203. [CrossRef]
42. Broncel M, Koziróg-Kołacińska M, Chojnowska-Jezierska J. Melatonin in the treatment of atherosclerosis. Pol Merkur Lekarski 2007; 23: 124-127.
43. Kesavan M, Sarath TS, Kannan K, Suresh S, Gupta P, Vijayakaran K, et al. Atorvastatin restores arsenic-induced vascular dysfunction in rats: modulation of nitric oxide signaling and inflammatory mediators. Toxicol Appl Pharmacol 2014; 280: 107-116. [CrossRef]
44. Baugh JA, Bucala R. Macrophage migration inhibitory factor. Crit Care Med 2002; 30: 27-35. [CrossRef]

45. Lue H, Kleemann R, Calandra T, Roger T, Bernhagen J. Macrophage migration inhibitory factor (MIF): mechanisms of action and role in disease. Microbes Infect 2002; 4: 449-460. [CrossRef]
46. Kobayashi S, Nishihira J, Watanabe S, Todo S. Prevention of lethal acute hepatic failure by antimacrophage migration inhibitory factor antibody in mice treated with bacille Calmette-Guerin and lipopolysaccharide. Hepatology 1999; 29: 1752-1759. [CrossRef]
47. Bourdi M, Reilly TP, Elkahloun AG, George JW, Pohl LR. Macrophage migration inhibitory factor in drug-induced liver injury: a role in susceptibility and stress responsivenessq. Biochem Biophys Res Commun 2002; 294: 225-230. [CrossRef]
48. Hoi AY, Iskander MN, Morand EF. Macrophage migration inhibitory factor: a therapeutic target across inflammatory diseases. Inflamm Allergy Drug Targets 2007; 6: 183-190. [CrossRef]