International Journal of
Medical Toxicology & Forensic Medicine

January 2024, Volume 14, Number 1

Research Paper

Hepatotoxic Effect of Hydrogen Cyanamide (Dormex®) in Albino Rats and the Ameliorative Effect of Melatonin

Amal Mohammed[*1], **Ahmed Salah El Sayed**[1]

1. Faculty of Medicine, Fayoum University, Faiyum, Egypt.

Use your device to scan and read the article online

Citation: Mohammed A, El Sayed AS. Hepatotoxic Effect of Hydrogen Cyanamide (Dormex®) in Albino Rats and the Ameliorative Effect of Melatonin. International Journal of Medical Toxicology and Forensic Medicine. 2024; 14(1):E43406. https://doi.org/10.32598/ijmtfm.v14i1.43406

https://doi.org/10.32598/ijmtfm.v14i1.43406

Article info:
Received: 27 Sep 2023
First Revision: 01 Oct 2023
Accepted: 25 Oct 2023
Published: 04 Feb 2024

Keywords:
Dormex®, Melatonin, Liver, Oxidative stress

ABSTRACT

Background: Hydrogen cyanamide (Dormex®) is used as a fertilizer sprayed on fruits, especially grapes to stimulate buds' opening. It causes oxidative stress leading to hepatic, renal, and lung damage. Melatonin is derived primarily from the amino acid tryptophan, produced from the pineal gland, and has antioxidant effects.

This study aimed to examine the effects of acute hydrogen cyanamide (Dormex®) exposure on the liver of albino rats and evaluate the biochemical and histological changes caused by Dormex® toxicity. Additionally, the study evaluated the potential ameliorative role of melatonin in these harmful effects.

Methods: Forty adult male albino rats were divided into four groups; group I: Negative control, group II: Melatonin-treated (100 mg/kg/day), group III: Dormex®-treated (100 mg/kg) as a single dose, and group IV: Receiving melatonin (100 mg/kg/day)+Dormex® (100 mg/kg). After 24 hours, all animals were evaluated for liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and bilirubin), hepatic markers for oxidative stress (malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD)) and histopathological examination was done for hepatic tissues.

Results: The Dormex®-treated group showed significantly elevated liver enzymes, elevated MDA, and decreased GSH and SOD. Histopathological examination revealed normal structure in groups 1 and 2 while group 3 showed several histopathological changes characterized by inflammation and hepatic necrosis. Administration of melatonin with Dormex® in group 4 caused a decrease in liver enzymes and MDA and an increase in GSH and SOD with improvement in liver histopathology.

Conclusion: Melatonin showed an ameliorative effect and can be used as a protective agent against Dormex®-induced hepatic injury.

*** Corresponding Author:**
Amal Mohammed, MD.
Address: *Faculty of Medicine, Fayoum University, Fayoum, Egypt.*
E-mail: *aml5raouf@hotmail.com*

Introduction

Hydrogen cyanamide (CH_2N_2) is utilized by certain countries as a nitrogen fertilizer in agriculture. Dormex®, which contains hydrogen cyanamide as its main active ingredient, is commonly sprayed on fruits, especially grapes, to stimulate bud opening and hasten the flowering process [1].

Dormex® can be exposed through ingestion, cutaneous contact, or inhalation. Any route has a quick absorption rate [2]. Dermal exposure causes severe inflammation as well as burns in the eyes and skin. Numerous experimental studies have revealed the toxic systemic effects of Dormex®, including the kidney, liver, blood, and thyroid gland [3].

The bioavailability of Dormex® can range from 45% to 80%, primarily influenced by the dosage administered [4]. Within the initial 24-hour period, approximately 40% of an orally administered dose is eliminated through the urine. N-acetylcyanamide is a primary metabolite eliminated in the urine. The remainder is either eliminated by stools or is eventually exhaled as carbon dioxide [1]. Dormex® is extremely hazardous to rats, with an oral LD_{50} of 300 mg/kg and a cutaneous LD_{50} of 1700 mg/kg [5].

Dormex®'s precise mode of toxicity is unknown. However, many studies suggested that Dormex® causes oxidative stress and uncoupling of oxidative phosphorylation [6]. Oxidative stress has been identified as a factor that can cause damage to several organs, including the liver, kidney, and lungs, as well as the central nervous system, cardiovascular system, and hemopoietic system [7].

Obvious liver toxicity was observed in many patients after acute Dormex® exposure with elevation of liver enzymes, prothrombin time, and bilirubin level. Some patients with acute hydrogen cyanamide poisoning have experienced a significant increase in serum creatinine [3, 8].

Melatonin (MEL), which is primarily derived from the amino acid tryptophan, is produced mainly from the pineal gland. It plays a crucial role in the regulation of various biological processes, such as the circadian rhythm, sleep, reproduction, and immunity [9].

Multiple studies have provided evidence of the diverse pharmacological actions exhibited by MEL, including antioxidant, anticancer, anti-inflammatory, antiapoptotic, and immunomodulatory properties [9, 10].

MEL demonstrates potent antioxidant activity and possesses protective properties against oxidative stress [11, 12].

Because of its ability to scavenge free oxygen radicals and protect cells and tissues from oxidative damage, MEL is the subject of several scientific studies [13]. In recent research, significant attention has been directed toward understanding the roles of MEL in lipid metabolism, oxidative stress, and its potential therapeutic applications. Multiple studies have demonstrated the effectiveness of melatonin in liver injuries and diseases.

This research aimed to study the hepatotoxic effect of acute exposure to hydrogen cyanamide (Dormex®) on albino rats and the protective effect of MEL.

Materials and Methods

Experimental animals

Forty adult male albino rats, weighing 200-230 g, were obtained from the National Research Center Breeding Unit (Giza, Egypt).

Before the experiment began, they were kept in standard laboratory rat conditions for one week and given free access to water and the recommended diet [14].

The rats were housed in clean plastic cages, with each cage accommodating five rats. To avoid any mixing, the name of the medication and the group number were clearly labeled on the outside of each cage. The rats were maintained in a clean and well-ventilated environment with regulated 12-hour light-dark cycles, a controlled temperature range of 21-24°C, and appropriate relative humidity levels.

Before the commencement of the experiment, animals underwent a 12-hour fasting period to ensure an empty stomach (with the allowance of water consumption only).

Chemicals

Dormex®

Dormex® (hydrogen cyanamide 50%) was produced by Alzchem group AG, Germany, and purchased from Trans Fridge International CO., Cairo, Egypt, in the form of a suspension of 5 liters.

Melatonin

MEL was produced by Sigma-Aldrich company and purchased from International CO., Cairo, Egypt for scientific and medical supplies.

Study design

The rats were divided into four groups with ten rats allocated to each group:

Group I: (Negative control group)

The rats were administered only saline.

Group II: (MEL group)

MEL was given at a dose of 100 mg/kg/day [15]. It was dissolved in a 1% ethanol solution and further diluted with normal saline [16].

Group III: (Hydrogen cyanamide group)

Dormex® was administered at a dosage of 100 mg/kg body weight (1/3 LD_{50}) suspended in 1 mL saline as a single dose via an oral cannula [5].

Group IV: (Melatonin+hydrogen cyanamide group)

Rats were received MEL (100 mg/kg/day) and hydrogen cyanamide (100 mg/kg)

Blood sampling & estimation of liver enzymes

After 24 hours, the animals in the various groups were anesthetized using sodium thiopental and euthanized. Blood samples were collected via cardiac puncture into non-heparinized containers and left to clot. After centrifugation at 1500 rpm for 15 minutes, the serum was separated and assessed for liver enzymes to determine Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) by colorimetric method using kits (Spectrum diagnostics company, Cairo, Egypt) and also serum bilirubin using ELIZA kits (BioVision Company, Cairo, Egypt) following the manufacturer's instruction.

Hepatic tissue preparation for histopathological examination

Animals were sacrificed by cervical decapitation after anesthesia. Laparotomy was carried out, and the livers were removed, washed with the ice-cold saline buffer, blotted in filter papers, and weighted. Then, the liver samples were fixed and stained with hematoxylin & eosin for histopathological study using the light microscope.

Estimation of markers of oxidative stress

One gram of hepatic tissue was taken from each rat and examined for malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD), by colorimetric method using kits (Biodiagnostic company, Giza, Egypt) following the manufacturer's instruction.

Statistical analysis

The collected data were presented as Mean±SD for all parameters. A one-way ANOVA was used to compare the statistical significance of the studied groups. $P \leq 0.05$ were considered statistically significant.

Results

Regarding tissue markers for oxidative stress, there was a significant increase in hepatic MDA levels in the hydrogen cyanamide-treated group (group III) compared to the control group.

Administration of MEL with hydrogen cyanamide in group IV was associated with a significant decrease in hepatic MDA levels in relation to group III.

There was a significant decrease in hepatic GSH and (SOD levels in the hydrogen cyanamide-treated group (group III) compared to the control group.

Administration of MEL with hydrogen cyanamide in group IV was associated with a significant increase in hepatic GSH and SOD levels in relation to group III (Table 1).

Regarding liver enzymes, there was a significant increase in serum levels of ALT, AST, ALP, and bilirubin in the hydrogen cyanamide-treated group (group III) compared to the control group.

Administration of MEL with hydrogen cyanamide in group IV showed a significant decrease in serum levels of liver enzymes compared to group III (Table 2).

Histopathology

Microscopic examination of the livers from both the control and MEL-treated groups (groups I & II) demonstrated a normal histological structure. The hepatocytes

Table 1. MDA, GSH, and SOD levels in the studied groups (n=10)

Variables	Group I	Group II	Group III	Group IV	Test Results
MDA (nmol/mg protein)	0.71±0.08	0.53±0.05	4.60±0.51	1.60±0.38	F=340.647 P<0.001
P1		0.606	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	
GSH (mmol/mg protein	4.10±0.30	4.85±0.30	1.28±0.21	2.79±0.25	F=350.322 P<0.001
P1		<0.001	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	
SOD (U/mg protein)	3.33±0.19	3.86±0.29	0.93±0.05	2.05±0.16	F=495.404 P<0.001
P1		<0.001	<0.001	< 0.001	
P2			<0.001	<0.001	
P3				<0.001	

International Journal of Medical Toxicology & Forensic Medicine

Note: F: One-way ANOVA, Statistically significant: P≤0.05, P1: Significance in relation to group I (control), P2: Significance in relation to group II (melatonin), P3: Significance in relation to group III (hydrogen cyanamide), Group I: -ve control, Group II: Melatonin, Group III: Hydrogen cyanamide, Group IV: Melatonin+hydrogen cyanamide.

were observed to be well-organized within the typical lobular architecture, with central veins and radiating hepatic cords. The portal triads exhibited a normal histological appearance, characterized by the presence of branches of the hepatic artery, hepatic portal vein, and bile duct (Figure 1 and 2).

Meanwhile, the hydrogen cyanamide-treated group (group III) showed several histopathological changes characterized by multifocal areas of mononuclear inflammatory cell infiltration associated with necrotic hepatocytes. The portal areas of some affected individuals showed numerous infiltrations of inflammatory cells accompanied by vacuolar degeneration of the adjacent hepatocytes (Figure 3).

Marked improvements were observed in the MEL+hydrogen cyanamide group (group IV), which showed apparently normal hepatic tissue in several examined sections, except for a few sections that showed fewer inflammatory cell infiltration and mild vacuolation of the existing hepatocytes (Figure 4).

Discussion

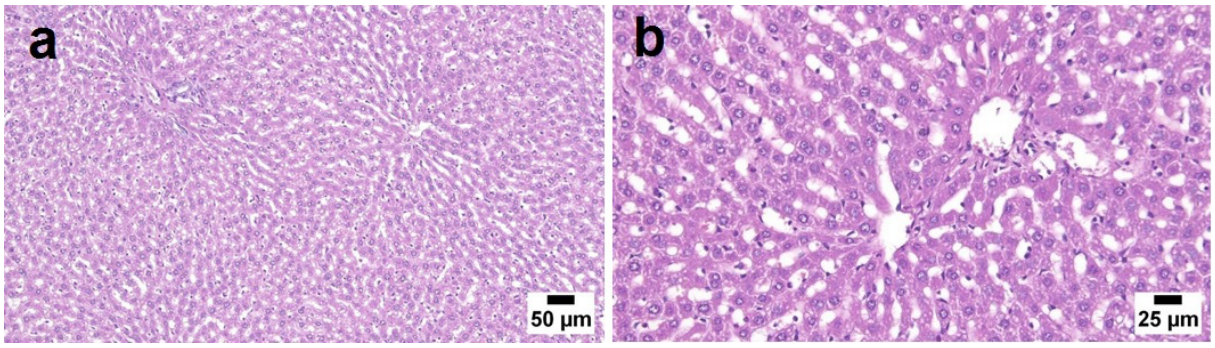


Figure 1. Liver tissue in the control group (group 1)

International Journal of Medical Toxicology & Forensic Medicine

a and b) Normal histological structure of hepatic lobules (H&E)

Table 2. ALT, AST, bilirubin, and ALP levels in the studied groups (n=10)

Variables	Group I	Group II	Group III	Group IV	Test Results
ALT (U/mL)	34.73±0.91	32.90±0.72	93.22±2.86	48.42±1.93	F=887.292 P<0.001
P1		0.133	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	
AST (U/mL)	48.34±0.37	45.62±0.39	106.69±5.41	60.64±0.63	F=971.620 P<0.001
P1		0.136	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	
Bilirubin (µg/mL)	9.04±0.49	9.18±0.40	57.38 ±1.26	26.42±0.54	F=923.606 P<0.001
P1		0.973	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	
ALP (ng/mL)	20.34±0.38	19.38±0.32	71.84±1.60	32.19±0.91	F=689.571 P<0.001
P1		0.131	<0.001	<0.001	
P2			<0.001	<0.001	
P3				<0.001	

Note: F: One-way ANOVA, Statistically significant: P≤0.05, P1: Significance in relation to group I (control), P2: Significance in relation to group II (melatonin), P3: Significance in relation to group III (hydrogen cyanamide), Group I: -ve control, Group II: Melatonin, Group III: Hydrogen cyanamide, Group IV: Melatonin+hydrogen cyanamide.

Around 1-5 million incidents of pesticide poisoning are estimated annually among agricultural workers, the majority of them happening in developing countries [17]. Dormex® is frequently used to control plant growth worldwide and in developing nations, like Egypt [7].

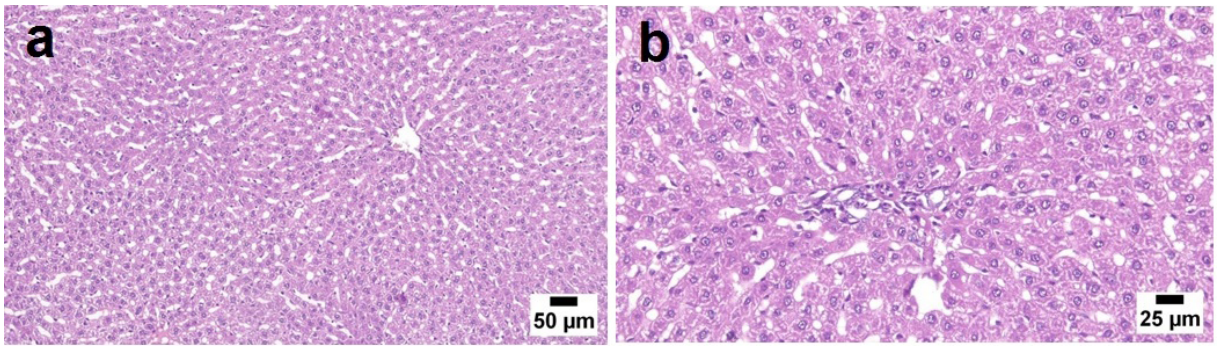
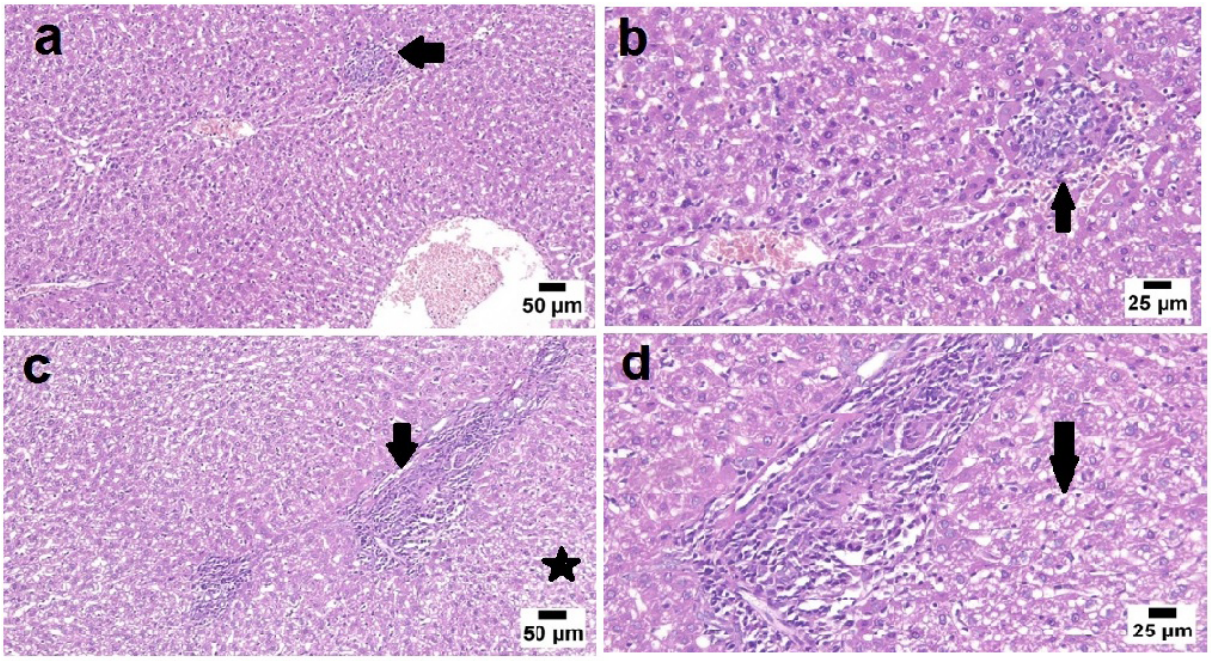


Figure 2. Liver tissue in the melatonin group (group 2)

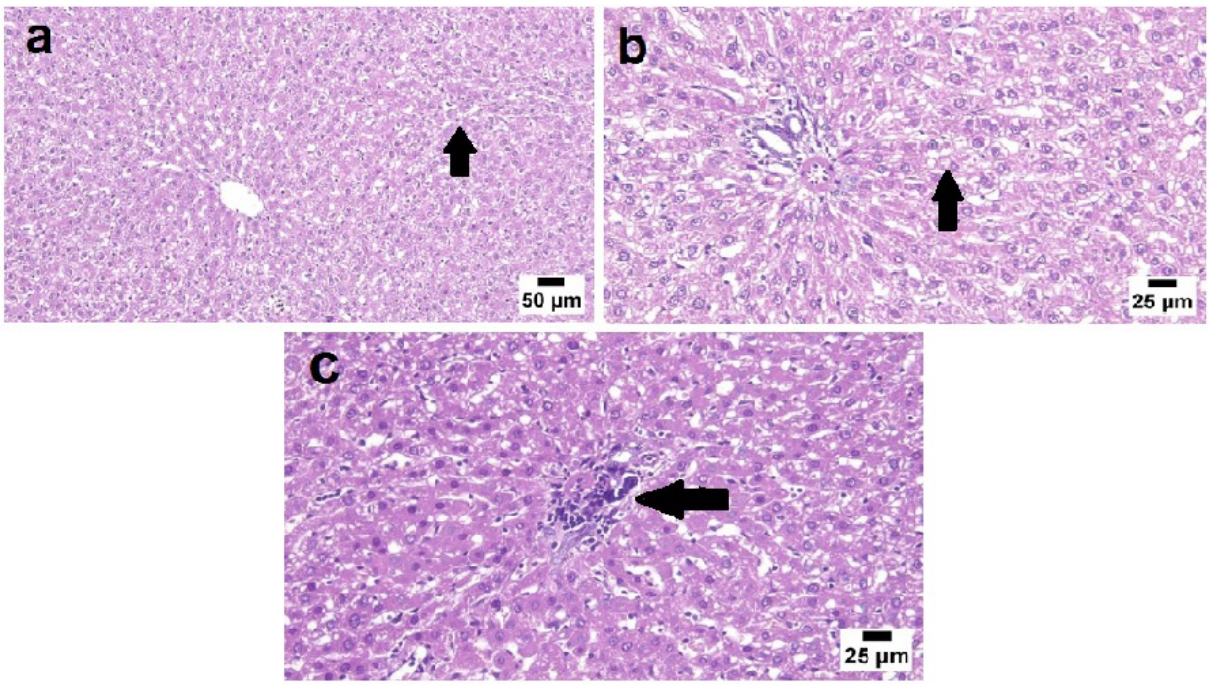
a) Normal histological structure of hepatic parenchyma (H&E), b) Normal portal area and intact adjacent hepatocytes (H&E)



International Journal of
Medical Toxicology & Forensic Medicine

Figure 3. Liver tissue in the hydrogen cyanamide group (group 3)

a) Infiltration of focal inflammatory cells with necrosis of the existing hepatocytes (arrow) (H&E), b) Infiltration of focal inflammatory cells with necrosis of the existing hepatocytes (arrow) (H&E), c) Portal hepatitis (arrow) with vacuolation of the adjacent hepatocytes (star) (H&E), d) Higher power showing portal hepatitis with vacuolation of the adjacent hepatocytes (arrow) (H&E)



International Journal of
Medical Toxicology & Forensic Medicine

Figure 4. Liver tissue of the melatonin+hydrogen cyanamide group (group IV)

a) Mild vacuolation of some hepatocytes (arrow) (H&E), b) Mild vacuolation of some hepatocytes (arrow) with healthy portal area (H&E), c) Mild portal inflammation (arrow) (H&E)

In contrast to how slowly it is absorbed through the skin, Dormex® is quickly absorbed from the gastrointestinal tract. When exposed, hydrogen cyanamide has both systemic and local effects. One of the postulated mechanisms of action is catalase enzyme inhibition, resulting in oxidative stress and uncoupling of oxidative phosphorylation [3].

The objective of the present study was to examine the biochemical and histological consequences of acute Dormex® poisoning on the liver, along with evaluating the potential protective effects of melatonin.

Regarding tissue markers for oxidative stress, the study observed a noteworthy elevation in hepatic MDA levels, indicating increased oxidative damage. Additionally, a substantial reduction in hepatic GSH and SOD levels in the hydrogen cyanamide-treated group was detected, suggesting compromised antioxidant defense mechanisms.

These results were consistent with a study that stated that oxidative stress parameters, like MDA, showed a significant increase in laboratory rats, while antioxidants, like glutathione reductase and catalase, were significantly reduced [18].

Similar results were observed by studying the effect of Dormax on kidneys, which showed a significant increase in MDA and a significant decrease in glutathione peroxidase in the group receiving Dormax [19].

The present study reported that the administration of MEL with hydrogen cyanamide in group IV was associated with a significant decrease in hepatic MDA levels and also, a significant increase in hepatic GSH and SOD.

These results agree with a study, which discovered that treatment with MEL slightly increased the activities of antioxidant parameters, like SOD, catalase, and GSH when compared with the CCl4-treated animals with hepatitis injury [20].

Also, our results are consistent with a previous study investigating the impact of MEL on aluminum-induced injury, which demonstrated that MEL treatment restored the balance between oxidant and antioxidant molecules. This was evidenced by a decrease in lipid peroxidation (LPO) and nitric oxide (NO) levels, as well as an increase in the activity of antioxidant enzymes and the content of GSH in the liver and kidney tissues [21].

MEL is a potent antioxidant and anti-inflammatory agent. Specifically, its aromatic indole ring acts as a buffer and scavenger for reactive oxygen and nitrogen species. Moreover, MEL has the capability to stimulate the production of endogenous antioxidant enzymes, such as catalase, SOD, glutathione peroxidase, and GSH. Additionally, MEL can inhibit the nuclear factor-kappa B (NFkB) transcription, which reduces the expression of inflammatory mediators, like cytokines [13, 22].

Regarding the liver function enzymes, the present study showed a significant rise in serum levels of ALT, AST, ALP, and bilirubin in the hydrogen cyanamide-treated group. This result is consistent with the study, which found that ALT and AST were significantly elevated in groups receiving doses of 100 and 300 mg/kg cyanamide [5]. Also, this result agrees with another study, which stated that the ingestion of Dormex® resulted in a substantial increase in serum levels of liver enzymes, including ALT, AST, ALP, and bilirubin in experimental rats [18].

ALT and AST enzymes are specific markers of hepatic injury. They reside in the cytoplasm of hepatocytes, and a spike in their serum activity indicates that they have escaped the cell as a result of altered cell membrane permeability, which ensures hepatocyte damage [23]. The increased total serum ALP is an indicator of hepatobiliary dysfunction and intrahepatic biliary cholestasis [24].

The present study noted that after the administration of MEL with hydrogen cyanamide, group IV showed a significant reduction in serum levels of liver enzymes. This result agrees with a study, which reported that ALT activity was decreased by 33.3% in the CCl4/MEL group in comparison with the CCl4 group. The AST activity was decreased by 29.5% compared to the CCl4 group [20].

Regarding the histopathology, the hydrogen cyanamide-treated group showed several histopathological changes characterized by multifocal areas of infiltration by mononuclear inflammatory cells associated with necrotic hepatocytes. The portal areas of some affected individuals showed infiltration of numerous inflammatory cells accompanied by vacuolar degeneration of the adjacent hepatocytes. This is consistent with the study, which noted that there was marked inflammatory cell infiltration associated with areas of vascular hemorrhages, and also, the central veins and portal vein branches were markedly dilated. Hepatocytes showing vacuolated cytoplasm were frequently seen in the 100 and 300 mg/kg cyanamide groups [5]. Also, another study noted that

Dormex®-treated groups showed hepatic inflammation, degeneration, and apoptosis [18].

The present study demonstrated the marked improvement of the MEL+hydrogen cyanamide group (group IV) that showed apparently normal hepatic tissue in several examined sections, except for a few sections that showed infiltration of fewer inflammatory cells and mild vacuolation of the existing hepatocytes. This result agreed with a study, which reported that the use of melatonin considerably inhibited aluminum-induced liver injury in rats. The study revealed that MEL treatment effectively restored the hepatic cell and lobular structures to a state close to normal. The findings suggest that MEL has the potential to prevent or reverse apoptosis, inflammation, and oxidative stress induced by aluminum in the liver and kidney tissues of rats [21].

Also, this result is consistent with a study, which observed that when MEL is given before the administration of thioacetamide (TAA), it caused partial tissue improvement, and when given after the application of TAA, the damage was greatly lessened. Histologically, the therapeutic effects of MEL on the tissue were more potent than its protective ones [25].

Upon contact with the cell membrane, MEL attaches to the outer surface of the phospholipid layer, intercepting free radicals before they reach the membrane and effectively neutralizing them. This mechanism allows MEL to protect the nucleus, organelles, and cell membrane from potential damage caused by free radicals [26, 27].

Conclusion

Hydrogen cyanamide triggers oxidative stress by an elevation in hepatic MDA and reduction in GSH and SOD levels also comprises liver enzymes and causes hepatic tissue inflammation and necrosis. MEL as an antioxidant has a hepatoprotective effect by counteracting these damaging effects.

Recommendations

Studying Dormex®-induced multi-organ toxicity is highly advised.

MEL can be used for the treatment of Dormex®-intoxicated patients.

Ethical Considerations

Compliance with ethical guidelines

The study received ethical approval from the Faculty of Medicine, Fayoum University (Code: R448) in compliance with the guidelines and regulations for the ethical treatment and use of laboratory animals. The minimum required number of animals was determined to ensure reliable and valid results.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interests.

References

[1] Oreby MM, Elgazzar FM, Hassab El-Nabi SE, Ali DA. The toxic effect of cyanamide (dormex) on testis in albino rats: Possible protective role of selenium. Egypt Journal of Forensic Science and Applied Toxicology. 2015; 15(2):205-23. [DOI:10.12816/0035688]

[2] Foti C, Bonamonte D, Conserva A, Pepe ML, Soleo L, Angelini G. Allergic contact dermatitis with a fertilizer containing hydrogen cyanamide (Dormex). Cutaneous and Ocular Toxicology. 2008; 27(1):1-3. [DOI:10.1080/15569520701598177] [PMID]

[3] El Mahdy NM, Kharoub HS. Acute toxicity due to occupational exposure to the plant growth regulator hydrogen cyanamide (case report). Egyptian Journal of Occupational Medicine. 2020; 44(1):471-84. [DOI:10.21608/ejom.2020.67632]

[4] Schep L, Temple W, Beasley M. The adverse effects of hydrogen cyanamide on human health: An evaluation of inquiries to the New Zealand National Poisons Centre. Clinical Toxicology (Philadelphia, Pa.). 2009; 47(1):58-60. [DOI:10.1080/15563650802459254] [PMID]

[5] Elmasry M, Osman HS, Mohammed A, Hady E. Cyanamide toxicity. Role of alpha tocopherol antioxidant. Ain-Shams Medical Journal. 2000; 51(4-6):457-77. [Link]

[6] Settimi L, Davanzo F, Travaglia A, Locatelli C, Cilento I, Volpe C, et al. [Italian program for surveillance of acute pesticide-related illnesses: Cases identified in 2005 (Italian)]. Giornale Italiano di Medicina del Lavoro ed Ergonomia. 2007; 29(3 Suppl):264-6. [PMID]

[7] Gamaluddin H, Mahmmoud S, Mosa M, Halawa H, Khalifa E. A clinical study of acute hydrogen cyanamide toxicity in the period between 2006-2009 in the poison control center, Ain Shams University. Ain Shams Journal of Forensic Medicine and Clinical Toxicology. 2012; 19:97-103. [DOI:10.21608/ajfm.2012.19540]

[8] Sharif AF, Fayed MM. Evaluation of multiple organ dysfunction score (MODS) and the sequential organ failure assessment (SOFA) score as in-hospital outcome predictors among cases of hydrogen cyanamide exposure: A cross-sectional study. Environmental Science and Pollution Research International. 2021; 28(31):42161-76. [DOI:10.1007/s11356-021-13655-6] [PMID]

[9] Fernández-Gil B, Moneim AE, Ortiz F, Shen YQ, Soto-Mercado V, Mendivil-Perez M, et al. Melatonin protects rats from radiotherapy-induced small intestine toxicity. Plos One. 2017; 12(4):e0174474. [DOI:10.1371/journal.pone.0174474] [PMID] [PMCID]

[10] Dutta S, Saha S, Mahalanobish S, Sadhukhan P, Sil PC. Melatonin attenuates arsenic induced nephropathy via the regulation of oxidative stress and inflammatory signaling cascades in mice. Food and Chemical Toxicology. 2018; 118:303-316. [DOI:10.1016/j.fct.2018.05.032] [PMID]

[11] Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, et al. Melatonin: An ancient molecule that makes oxygen metabolically tolerable. Journal of Pineal Research. 2015; 59(4):403-19. [DOI:10.1111/jpi.12267] [PMID]

[12] Bali İ, Bilir B, Emir S, Turan F, Yılmaz A, Gökkuş T, et al. The effects of melatonin on liver functions in arsenic-induced liver damage. Ulusal Cerrahi Dergisi. 2016; 32(4):233-7. [DOI:10.5152/UCD.2015.3224] [PMID] [PMCID]

[13] Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: Under promises but over delivers. Journal of Pineal Research. 2016; 61(3):253-78. [DOI:10.1111/jpi.12360] [PMID]

[14] Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition. 1993; 123(11):1939-51. [DOI:10.1093/jn/123.11.1939] [PMID]

[15] Koksal M, Kurcer Z, Erdogan D, Iraz M, Tas M, Eren MA, et al. Effect of melatonin and n-acetylcysteine on hepatic injury in rat induced by methanol intoxication: A comparative study. European Review for Medical and pharmacological Sciences. 2012; 16(4):437-44. [PMID]

[16] Kaplan M, Atakan IH, Aydoğdu N, Aktoz T, Puyan, FO, Şeren G, et al. The effect of melatonin on cadmium-induced renal injury in chronically exposed rats. Turkish Journal of Urology. 2009; 35(2):139-47. [Link]

[17] Abdou KA, Abo El-Atta HM. Epidemiology of pesticides in developing countries. Advances in Clinical Toxicology. 2018; 3(1):1-8. [DOI:10.23880/ACT-16000128]

[18] Elhosary N, Elgharbawy DE, Kassab A, Elgharabawy RM. Cyanamide-induced hepatotoxicity and the potential protective role of pomegranate seed extract in adult male albino rats. Ain Shams Journal of Forensic Medicine and Clinical Toxicology. 2018; 30(1):38-51. [DOI:10.21608/ajfm.2018.18135]

[19] Elbastawesy S, Ibrahim F, Abo Safia H, El Shamy A, Ibrahim H, Helal N. Nephrotoxic effect of hydrogen cyanamide (dormex) and possible protective role of n-acetyl cysteine in male albino rats. Ain Shams Journal of Forensic Medicine and Clinical Toxicology. 2022; 39(2):21-9. [DOI:10.21608/ajfm.2022.244112]

[20] Oleshchuk O, Ivankiv Y, Falfushynska H, Mudra A, Lisnychuk N. Hepatoprotective effect of melatonin in toxic liver injury in rats. Medicina. 2019; 55(6):304. [DOI:10.3390/medicina55060304] [PMID] [PMCID]

[21] Othman MS, Fareid MA, Abdel Hameed RS, Abdel Moneim AE. The protective effects of melatonin on aluminum-induced hepatotoxicity and nephrotoxicity in rats. Oxidative Medicine and Cellular Longevity. 2020; 2020:7375136. [DOI:10.1155/2020/7375136] [PMID] [PMCID]

[22] Ferlazzo N, Andolina G, Cannata A, Costanzo MG, Rizzo V, Currò M, et al. Is melatonin the cornucopia of the 21st century? Antioxidants. 2020; 9(11):1088. [DOI:10.3390/antiox9111088] [PMID] [PMCID]

[23] Rosen HR, Keeffe EB. Evaluation of abnormal liver enzymes, use of liver test and the serology of viral hepatitis. In: Bacon B, Bisceglie AM, editors. Liver disease diagnosis and management. New York: Churchill Livingstone; 2000.

[24] Saxena B, Sharma S. Food color induced hepatotoxicity in swiss albino rats, rattus norvegicus. Toxicology International. 2015; 22(1):152-7. [DOI:10.4103/0971-6580.172286] [PMID] [PMCID]

[25] Sayan M, Karabulut D, Özdamar S. Assessment of the protective and therapeutic effect of melatonin against thioacetamide-induced acute liver damage. Journal of Biochemical and Molecular Toxicology. 2020; 34(4):e22450. [DOI:10.1002/jbt.22450] [PMID]

[26] Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Alméras N, et al. Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? Circulation. 2000; 102(2):179-84. [DOI:10.1161/01.CIR.102.2.179] [PMID]

[27] Koziróg M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, Broncel M. Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. Journal of Pineal Research. 2011; 50(3):261-6. [DOI:10.1111/j.1600-079X.2010.00835.x] [PMID]