Saudi Journal of Biological Sciences 29 (2022) 103425

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Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Toxic effects of excess exposure to boric acid on serum biochemical aspect, hematology and histological alterations and ameliorative potential role of melatonin in rats

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ARTICLE INFO

Article history:
Received 11 May 2022
Revised 16 July 2022
Accepted 17 August 2022
Available online 22 August 2022

Keywords:
Ameliorative role
Biochemical assay
Boric acid
Hematology
Histopathology
Melatonin

ABSTRACT

The current work clarifies the negative effects of excess exposure to boric acid (H_3BO_3) as a boron-containing compound on rats and the possible ameliorative effect of melatonin (MEL). Forty rats were equally divided into 5 groups as follows: group 1 was treated as control while groups 2, 3, 4 and 5 were orally administered corn oil (0.5 ml), H_3BO_3 (1330 mg/kg BW), MEL (10 mg/kg BW) and H_3BO_3 + MEL for 28 consecutive days, respectively. At the end of the experiment, blood was sampled for biochemical and hematological analysis and tissues were collected for histopathological examination. The obtained results demonstrated that the exposure to H_3BO_3 induced hepatorenal dysfunctions, alterations in bone-related minerals and hormones levels, prostaglandin E2 as inflammatory mediator and hematological indices. H_3BO_3 induced histological alterations in the liver, kidneys, bone and skin. The co-administration of MEL with H_3BO_3 resulted in a significant improvement in most of the measured parameters and restoration of morpho-functional state of different organs compared to the H_3BO_3 group. In conclusion, the study clearly demonstrated that H_3BO_3- induced various adverse effects and that melatonin may be beneficial in a partial mitigating the H_3BO_3 and may represent a novel approach in the counteracting its toxicity.

1. Introduction

Boron (B) is a unique and significant element that does not occur in the elemental form, but it exists in nature as borates and boric acid (Woods, 1994; Scorei, 2012). Boric acid (BA) has been essential for plants and animals and beneficial for humans at nutritional levels, where it is taken from water, vegetables and many other foodstuffs. Also, it used in many manufacturing processes (Howe, 1998; Nielsen, 2008).

Boric acid (H_3BO_3) is used in nutritional supplements as a source of boron and as a food preservative in some food products. Consuming it in high amounts is harmful to human health. Still, it is used in the food production process, especially in noodles and some processed seafood such as fish ball, despite the risk of boric acid (Miggiano and Gagliardi, 2005; Yiu et al., 2008). Boric acid was enormously used in medicine at the beginning of the century for therapeutic purposes as an antimicrobial preservative in eye drops, ointments and topical creams; as suppositories to treat yeast infection and in dilute concentrations as a mild antiseptic (Rowe et al., 2006). Boric acid is also applied as an herbicide, where it acts to disrupt the photosynthesis system in plants, so it is often used to repress algae in sewage systems and swimming pools (Cox, 2004). It also plays a role as an insecticide (stomach poison) for some pest such as ants, termites and cockroaches (Habes et al., 2013; Kafle et al., 2020).

Ambient environment (air, water or soil) contamination by boron can occur from both natural and anthropogenic sources, especially with the increase in the application of boric acid in industrial and other related fields (Akar, 2007; Xu et al., 2010).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, calcium; CT, calcitonin; GGT, gamma-glutamyl transferase; H_3BO_3, boric acid; Hb, hemoglobin; Ht, hematocrit; MEL, melatonin; Mg, magnesium; P, phosphorus; PGE2, prostaglandin E2; PTH, parathyroid hormone; RBCs, red blood cells; WBCs, White blood cells.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103425
1319-562X/

Also, boric acid toxicity may occur if improperly used (See et al., 2010).

Melatonin (MEL) is a product of pineal gland in vertebrates, which once synthesized, is not stored in pineal cells, but it is released rapidly into the blood circulation (Tan et al., 2003). Nowadays, melatonin is of a special interest because of its universal protective role against any stress of chemical origin for humans and animals. The antitoxic activity of melatonin, besides its clear immunotropic characters, shed the light on the need for further study of it as a potential agent to combat intoxication effects of different chemicals. These findings, besides the fact that melatonin has no side effects or toxicity of its own, may prove a rather valuable chemical substance to use in the toxicology field (Khodzhayan et al., 2014). Melatonin is an efficient free radical scavenger, where it can enter each cell of the body due to its distinct physical and chemical properties and this gives it the ability to cross all morpho-physiological barriers and stimulate various antioxidant enzymes for protecting molecules from oxidative damage in both aqueous and lipid environments of the cell (Tan et al., 2003; Rana, 2018). Melatonin also plays an important role in the detoxification of xenobiotics and endobiotic compounds through pleiotropic mechanisms (Rana, 2018) and has a pro-inflammatory role in early inflammation and an anti-inflammatory role in late inflammation process (Radogna et al., 2010).

The current work aims to clarify the negative effects of excess exposure to boric acid as a boron-containing compound on rats and the possible ameliorative effect of melatonin. For this purpose, the assessment of hepatorenal function-related parameters, serum proteins profile, bone-related minerals and hormones and hematological variables were done, in addition to the histopathological examination of different tissues to confirm the findings.

2. Materials and methods

2.1. Tested chemicals and animals

Boric acid (CAS-No.: 10043-35-3) used in this study was a white powder with purity ≥99.8 %. Melatonin (CAS-No.: 73-31-4) used in this study was white to off-white powder with purity ≥98 %. Both chemicals were bought from Sigma-Aldrich Chemie GmbH, Germany. The corn oil was purchased from the agricultural research center, Giza, Egypt.

Forty male adult Wistar rats (155–175 g) were obtained from the laboratory animal house in the Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. Rats were separated in standard cages and kept on a standard diet and given tap water *ad libitum* in addition to maintain the standard conditions (25 ± 1 °C, 45 ± 5 % relative humidity and 12-h light–dark cycle). Animals acclimated for one week before starting the experiment. The health conditions of the animals were monitored daily.

2.2. Experimental protocol

Experimental rats (n = 40) were randomly split into five groups of eight animals each. Group 1 served as the control group; group 2 received corn oil (0.5 ml; vehicle group); group 3 (H_3BO_3 group) received boric acid at a dose of 1330 mg/kg BW (1/2 LD_{50} oral), keeping in the mind that (LD_{50} oral) for the rat was 2660 mg/kg BW according to Bingham and Cohrssen (2012); group 4 (MEL group) received melatonin at a dose of 10 mg/kg BW, which was supported by earlier studies (Bhatti et al., 2014; El-Gerbed, 2017) and group 5 (H_3BO_3 + MEL group) received melatonin then boric acid in the same doses, route and duration of groups 3 and 4. Boric acid and melatonin were suspended in corn oil. Rats were exposed to the tested agents orally through the esophageal tube for 28 consecutive days.

2.3. Blood and tissue sample

At the end of the experimental period, blood samples (n = 6/-group) were obtained by puncturing the *retro*-orbital venous sinus from overnight fasted animals that anesthetized with sodium pentobarbital (50 mg/kg BW), intraperitoneal. Collected blood was immediately placed into two tubes; the first fraction (1.5 ml) was transferred into a plain tube and allowed to stand for 30 min for blood clotting, then centrifuged to obtain the serum and maintained at −20 °C till performing the clinical chemistry tests. The second fraction (1 ml) was transferred to an ethylenediaminetetraacetic acid (EDTA) tube to get whole blood for hematological studies. Experimental animals (n = 6/group) were euthanized after anesthesia by decapitation and the tissues of liver, kidneys, bone (tibia) and skin were quickly taken away for histopathological study.

2.4. Determination of serum clinical chemistry biomarkers

The activities of alanine aminotransferase (ALT; Ref: 1001170), aspartate aminotransferase (AST; Ref: MD41264), gamma-glutamyl transferase (GGT; Ref: MD41288), alkaline phosphatase (ALP; Ref: MD41233) as well as the levels of creatinine (Ref: MD1001111), urea (Ref:1001332), total proteins (Ref: MD1001291), calcium (Ca; Ref: MD1001065), phosphorus (P; Ref: Mx100115 5) and magnesium (Mg; Ref: TK1001285) were assessed in serum by the methods of Tietz (1995); Burtis and Ashwood (1999) and Friedman and Young (2001). All these biomarkers were measured using specific commercial diagnostic kits following the manufacturer's instructions and purchased from Spinreact, Spain. These parameters were determined using semi-auto chemistry analyzer (Chem-7) manufactured by Erba Diagnostics, Germany; Electrolyte Analyzer-EA205 manufactured by NS Biotec, Egypt and ADVIA 1800 chemistry system manufactured by Siemens, Japan.

Serum proteins fractionation was done using a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) technique for the albumin and alpha (α), beta (β) and gamma (γ) globulins according to the method clarified by Davis (1964) and Ornstein (1964).

Parathyroid hormone (PTH; Catalogue No. CSB-E07866r), calcitonin hormone (CT; Catalogue No.: E05132r) and prostaglandin E2 (PGE2; Catalogue No.: CSB-E07967r) were assayed using ELISA kits purchased from Cusabio according to the kit's guidelines. The technique for measuring these parameters is the quantitative sandwich enzyme immunoassay. The principle of the assay depends on the pre-coating of the microplate by antibodies-specific for tested parameters. PTH, CT and PGE2 that were present in the sample were bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for tested parameters was added to the wells, followed by a washing step, and then avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Any unbound avidin-enzyme reagent was removed by washing and then a substrate solution was added to the wells and color developed in proportion to the amount of tested parameter bound in the initial step. The color development was stopped, and the color intensity was measured. Also, testosterone (Catalogue No.: EK7014) and 17-beta estradiol (17β-estradiol; Catalogue No.: LS-F67094) were measured using an ELISA kit purchased from Boster Biological Technology and Lifespan Biosciences, respectively, according to the kits guidelines. The measurement of serum hormones occurred at 450 nm using

the RT-2100C microplate reader (Rayto Life and Analytical Sciences, China).

2.5. Determination of hematological parameters

The parameters analyzed included red blood cells (RBCs) as well as total and differential white blood cells (WBCs) counts, hemoglobin (Hb) concentration and hematocrit (Ht) value. These parameters were estimated using an automated blood cell counter (Sysmex XT-2000iV, Kobe, Japan; Buttarello and Plebani, 2008).

2.6. Histopathological examination and semi-quantitative scoring system

The collected liver, kidneys, bone (tibia) and skin tissue specimens were kept in 10 % neutral buffered formalin for fixation process then dehydrated in ascending degrees of alcohol, after that cleared in xylene, then paraffinized and sectioned into 5-µm thin pieces. Lastly, the prepared tissue sections were mounted on glass slides and stained with hematoxylin and eosin (H&E; Suvarna et al., 2018). All slides were examined to assess any histological alterations in different tissues and photomicrographs were captured using a light microscope. Semi-quantitative scoring of histopathological alterations was evaluated to express the degree of severity of the different histopathological alterations observed in the examined tissues in this study of rat groups (Gibson-Corley et al., 2013). Each grade revealed histological characteristics and was classified: (−) no histological alterations, which showed normal histological structure; (+/−) fine to mild histopathological changes, (+1) mild histopathological changes, (+2) moderate histopathological changes and (+3) severe histopathological changes.

2.7. Data analysis and statistical procedures

The gathered data from the current study were analyzed using SPSS software (version 21, IBM Corporation, Armonk, NY, USA) and subjected to statistical processing using the method of one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (Snedecor and Cochran, 1994). Data from different groups were represented as means ± standard errors (SE) and a significant change was considered if $p < 0.05$. The results were represented graphically using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Serum clinical chemistry biomarkers

3.1.1. Some hepatorenal parameters

Results showed a significant ($p < 0.001$) decrease in serum ALT and ALP activities and an increase in serum GGT activity in groups exposed to H_3BO_3 and H_3BO_3 + MEL compared to the control group. Cotreatment of H_3BO_3 with MEL significantly diminished the changes in the activities of these enzymes as compared to the H3BO3-exposed rats. However, serum AST activity showed a non-significant change in experimental groups in comparison to the control group. Compared to the control group, serum creatinine level showed a significant ($p = 0.002$) increase in the H_3BO_3-exposed group and an insignificant increase in the H_3BO_3 + MEL group, while serum urea level showed a significant ($p < 0.001$) decrease and increase in H_3BO_3 and H_3BO_3 + MEL groups, respectively. However, the administration of MEL alone showed a non-significant change in all these parameters in comparison to the control group as well as the vehicle group (Table 1).

3.1.2. Serum proteins profile

Results revealed a significant decrease in serum total proteins ($p = 0.001$) and albumin ($p < 0.001$) concentrations in both groups exposed to H_3BO_3 and H_3BO_3 + MEL compared to the control group. The marked reduction in these parameters was noticed in rats exposed to H_3BO_3 in comparison to group co-administered H_3BO_3 and MEL. Serum α1-globulin showed a significant increase ($p = 0.002$) in rats that received H_3BO_3 alone compared to the control. The administration of MEL alone as well as the vehicle group did not induce any significant change in these parameters. Non-statistical significant differences were observed in serum globulins ($p = 0.529$) and α2 ($p = 0.101$), β ($p = 0.327$) and γ ($p = 0.456$) globulin levels in all experimental groups (Table 2).

3.1.3. Some key minerals in bone metabolism

Serum Ca and Mg levels significantly ($p < 0.001$) increased in groups exposed to H_3BO_3 and H_3BO_3 + MEL compared to the control group. The values of serum Ca and Mg declined in rats co-administered the MEL with H_3BO_3 in comparison to group exposed to H_3BO_3 alone. However, serum P level significantly decreased in comparison to the control group in H_3BO_3 and H_3BO_3 + MEL groups. Compared to H_3BO_3-exposed group, the values of this parameter significantly improved in the H_3BO_3 + MEL treated group. MEL treatment alone as well as vehicle group did not cause any significant alterations in these parameters (Fig. 1).

3.1.4. Some key hormones for bone metabolism and prostaglandin E2

Serum CT, testosterone and 17-beta estradiol hormones as well as PGE2 levels were significantly increased ($p < 0.001$) in H_3BO_3 and H_3BO_3 + MEL groups compared to the control. Serum PTH level significantly reduced ($p < 0.001$) in H_3BO_3 and H_3BO_3 + MEL groups compared to the control group. Moreover, the marked alterations, either increase or decrease of these parameters were observed in the H_3BO_3 treated group in comparison to the H_3BO_3 + MEL treated group. Rats that received MEL or vehicle in this study showed non-significant changes in these parameters in comparison to the control group (Fig. 2).

3.2. Hematological parameters

Data revealed a significant elevation ($p < 0.001$) in RBCs count, Hb concentration and Ht value in rat groups that received H_3BO_3 and H_3BO_3 + MEL compared to the control and the highest values were observed in group received H_3BO_3 alone in comparison to the H_3BO_3 + MEL treated group. Leukopenia accompanied with neutropenia, lymphopenia and monocytopenia were observed in groups administered with H_3BO_3 and H_3BO_3 + MEL compared to the control group ($p < 0.001$). The lowest values were observed in the H_3BO_3-exposed group in comparison to the H_3BO_3 + MEL treated group. Eosinophils and basophils counts showed non-statistical significant difference in all tested groups ($p = 0.690$) and ($p = 0.072$), respectively. Rats in the vehicle group and MEL-administered group did not show any significant change in erythrogram and leukogram parameters compared to the control (Table 3).

3.3. Histopathological examination and semi-quantitative scoring system

Light microscopic evaluation of rats' liver sections of each control (Fig. 3A), vehicle (Fig. 3B) and MEL (Fig. 3F) groups revealed normal hepatic architectures including normal lobules, central vein, sinusoids, hepatic cords, portal triads and Kupffer cells. In contrast, examined sections from rats that received H_3BO_3 (Fig. 3-C-E) showed a marked widening of blood vessels besides multifocal necrotic areas. The necrotic hepatic parenchyma was replaced

Table 1
Some hepatorenal biochemical parameters after 28 days of starting the experiment in different rat groups.

Parameters	Control	Animal groups				
		Vehicle	H_3BO_3	MEL	H_3BO_3 + MEL	p-value
ALT (U/L)	79.38 ± 0.954^{ab}	78.17 ± 0.274^{b}	57.43 ± 0.981^{d}	81.49 ± 0.636^{a}	65.59 ± 0.702^{c}	<0.001
AST (U/L)	153.72 ± 0.807^{ab}	154.16 ± 0.354^{ab}	155.44 ± 0.258^{a}	155.56 ± 0.362^{a}	152.68 ± 0.511^{b}	0.003
GGT (U/L)	5.66 ± 0.003^{c}	5.69 ± 0.024^{c}	9.06 ± 0.041^{a}	5.54 ± 0.065^{c}	7.09 ± 0.024^{b}	<0.001
ALP (U/L)	225.76 ± 2.046^{ab}	230.80 ± 1.418^{a}	90.51 ± 1.181^{d}	222.78 ± 1.035^{b}	139.26 ± 1.183^{c}	<0.001
Creatinine (mg/dl)	0.71 ± 0.015^{b}	0.72 ± 0.013^{b}	0.82 ± 0.029^{a}	0.71 ± 0.009^{b}	0.75 ± 0.103^{ab}	0.002
Urea (mg/dl)	37.22 ± 0.801^{b}	38.16 ± 1.018^{b}	27.15 ± 0.498^{c}	38.14 ± 0.885^{b}	74.18 ± 0.973^{a}	<0.001

Data are expressed as mean ± SE. ^{abcd} Mean values within same row not sharing a common superscript letter were significantly different ($p < 0.05$), and the highest value was represented by the letter (a), same letters indicate no differences. ALT-alanine aminotransferase; AST-aspartate aminotransferase; GGT-gamma-glutamyl transferase; ALP-alkaline phosphatase.

Table 2
Serum proteins profile after 28 days of starting the experiment in different rat groups.

Parameters	Control	Animal groups				
		Vehicle	H_3BO_3	MEL	H_3BO_3 + MEL	p-value
Total proteins (g/dl)	6.40 ± 0.200^{a}	6.23 ± 0.033^{ab}	5.13 ± 0.166^{c}	6.20 ± 0.251^{ab}	5.50 ± 0.100^{bc}	0.001
Albumin (g/dl)	3.73 ± 0.088^{a}	3.47 ± 0.033^{a}	2.43 ± 0.033^{c}	3.50 ± 0.100^{a}	3.07 ± 0.016^{b}	<0.001
Globulins (g/dl)	2.67 ± 0.202	2.76 ± 0.033	2.70 ± 0.152	2.70 ± 0.152	2.43 ± 0.092	0.529
α1-globulin (g/dl)	0.65 ± 0.086^{b}	0.75 ± 0.028^{b}	1.07 ± 0.066^{a}	0.77 ± 0.033^{b}	0.73 ± 0.016^{b}	0.002
α2-globulin (g/dl)	0.75 ± 0.031	0.75 ± 0.028	0.60 ± 0.100	0.75 ± 0.028	0.55 ± 0.076	0.101
β-globulin (g/dl)	0.88 ± 0.044	0.83 ± 0.044	0.70 ± 0.057	0.80 ± 0.100	0.78 ± 0.016	0.327
γ-globulin (g/dl)	0.39 ± 0.041	0.43 ± 0.015	0.33 ± 0.033	0.38 ± 0.044	0.37 ± 0.033	0.456

Data are expressed as mean ± SE. ^{abc}Mean values within same row not sharing a common superscript letter were significantly different ($p < 0.05$), and the highest value was represented by the letter (a), same letters indicate no differences. No letters indicate ($p > 0.05$).

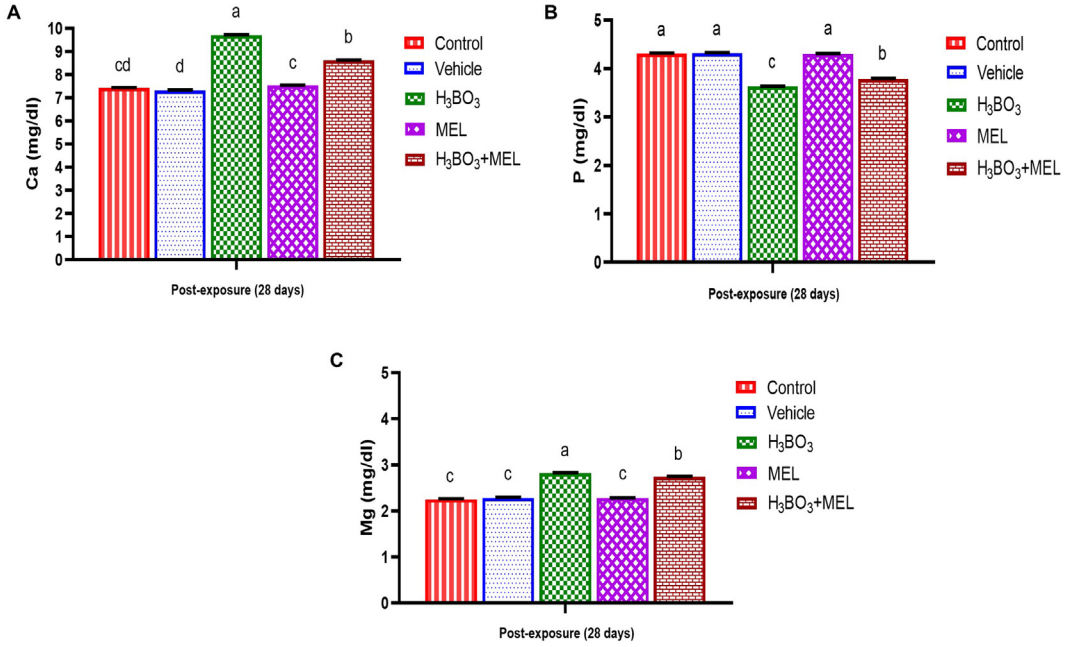

Fig. 1. Serum calcium, phosphorus and magnesium levels after 28 days of starting the experiment in different rat groups. Data are expressed as mean ± SE. ^{abcd} Columns not sharing a common superscript letter are significantly different ($p < 0.001$) and the highest value was represented by the letter (a), same letters indicate no differences. Ca-calcium; P-phosphorus; Mg-magnesium.

with chronic inflammatory reaction, including epithelioid histiocytes admixed with chronic round cells. The portal area suffered from perivascular inflammatory cells infiltrations with congested blood vessels. Also, prominent peribiliary and portal fibrosis and cholangitis were seen. Meanwhile, examined sections from rats' liver received H_3BO_3 + MEL revealed several forms of remodeling signs represented by returning the hepatic parenchyma to nearly normal hepatocytes with still minute inflammatory cells infiltrations the portal areas, besides mild congested blood vessels and prominent hypertrophied Kupffer cells. (Fig. 3G,H).

Microscopic examination of kidney sections from rats in the control (Fig. 4A), vehicle (Fig. 4B) and MEL (Fig. 4F) groups revealed normal renal cortex structures within normal glomerular tufts, glomerular space and its capsule followed by normal proximal renal tubules epithelium and interstitial tissue structures as well as medulla histological structures. However, kidney sections from rats exposed to H_3BO_3 revealed multifocal interstitial hemorrhage,

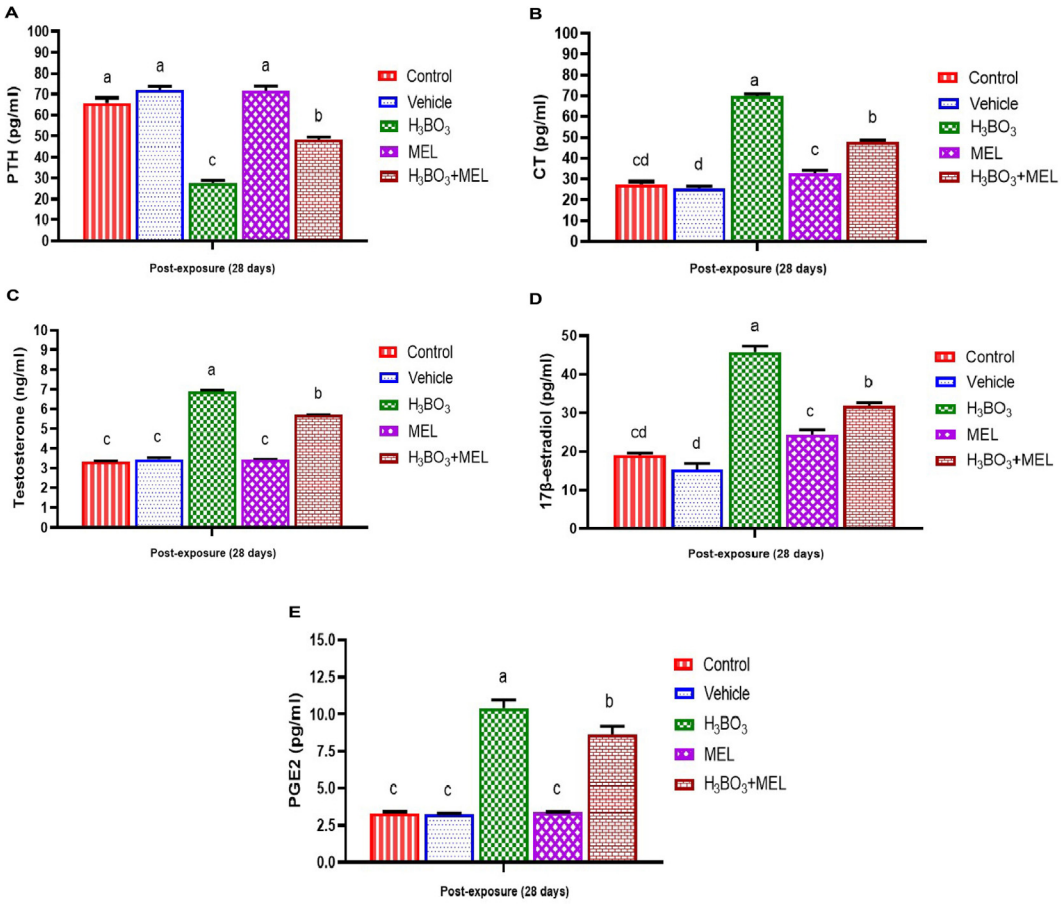

Fig. 2. Serum levels of parathyroid, calcitonin, testosterone and 17-beta estradiol hormones and prostaglandin E2 after 28 days of starting the experiment in different rat groups. Data are expressed as mean ± SE. [abcd] Columns not sharing a common superscript letter are significantly different ($p < 0.001$) and the highest value was represented by the letter (a), same letters indicate no differences. PTH-parathyroid hormone; CT-calcitonin; PGE2- prostaglandin E2.

Table 3
Hematological markers after 28 days of starting the experiment in different rat groups.

Parameters	Control	Animal groups				
		Vehicle	H_3BO_3	MEL	H_3BO_3 + MEL	p-value
RBCs ($\times 10^6/\mu l$)	5.89 ± 0.100[c]	5.84 ± 0.083[c]	7.50 ± 0.063[a]	5.89 ± 0.082[c]	6.84 ± 0.127[b]	<0.001
Hb (g %)	11.99 ± 0.162 [cd]	11.28 ± 0.288[d]	16.27 ± 0.186[a]	12.37 ± 0.163[c]	14.94 ± 0.165[b]	<0.001
Ht (%)	34.21 ± 0.695[c]	34.65 ± 0.168[c]	41.30 ± 0.082[a]	33.47 ± 1.073[c]	37.97 ± 0.581[b]	<0.001
WBCs ($\times 10^3/\mu l$)	12.58 ± 0.084[a]	12.28 ± 0.130[a]	5.49 ± 0.216[c]	11.90 ± 0.178[a]	8.04 ± 0.249[b]	<0.001
Neutrophils ($\times 10^3/\mu l$)	1.49 ± 0.051[a]	1.40 ± 0.080[a]	0.61 ± 0.022[c]	1.35 ± 0.065[a]	0.92 ± 0.027[b]	<0.001
Lymphocytes ($\times 10^3/\mu l$)	10.39 ± 0.125[a]	10.14 ± 0.054[a]	4.67 ± 0.193[c]	9.88 ± 0.117[a]	6.77 ± 0.216[b]	<0.001
Monocytes ($\times 10^3/\mu l$)	0.65 ± 0.019[a]	0.68 ± 0.018[a]	0.16 ± 0.006[c]	0.62 ± 0.004[a]	0.29 ± 0.019[b]	<0.001
Eosinophils ($\times 10^3/\mu l$)	0.03 ± 0.002	0.04 ± 0.001	0.03 ± 0.004	0.04 ± 0.003	0.04 ± 0.004	0.690
Basophils ($\times 10^3/\mu l$)	0.02 ± 0.002	0.02 ± 0.002	0.02 ± 0.002	0.01 ± 0.002	0.02 ± 0.002	0.072

Data are expressed as mean ± SE. [abc]Mean values within same row not sharing a common superscript letter were significantly different ($p < 0.05$), and the highest value was represented by the letter (a), same letters indicate no differences. No letters indicate ($p > 0.05$). RBCs-red blood cells; Hb-hemoglobin; Ht-hematocrit; WBCs-White blood cells.

congested blood vessels, necrotic renal tubules and glomeruli among massive interstitial round cells (chronic inflammatory reaction including active fibroblast and/or chronic round cells) and/or fibroblast proliferations, besides injured renal tubules epithelium, which sometimes contains granular casts. Other renal sections suffered from engorged blood vessels surrounded by edema followed with injured renal tubules, which contained casts besides lobulated glomerular tufts (Fig. 4C-E). Regarding evaluation sections from rats received H_3BO_3 + MEL revealed nearly normal renal parenchymal structures with mildly appearance of cystic dilatation of many renal tubules and a few granular casts with still lobulated a few glomeruli and sometimes extravasated erythrocytes (Fig. 4 G, H).

The histological examination of the all-bone sections from control (Fig. 5A), vehicle (Fig. 5B) and MEL (Fig. 5G) groups showed normal compact bone as an osteocyte inside the lacunae and blood vessels inside the Haversian canal among a homogenous eosinophilic stained matrix within the normal periosteum and endosteum followed by normal bone marrow structure. Furthermore, sections of bone from rats exposed to H_3BO_3 revealed osteoclastic reactivity in the hyper-cellularity periosteum layer (active osteoclastic admixed with epithelized histiocytes and some debris

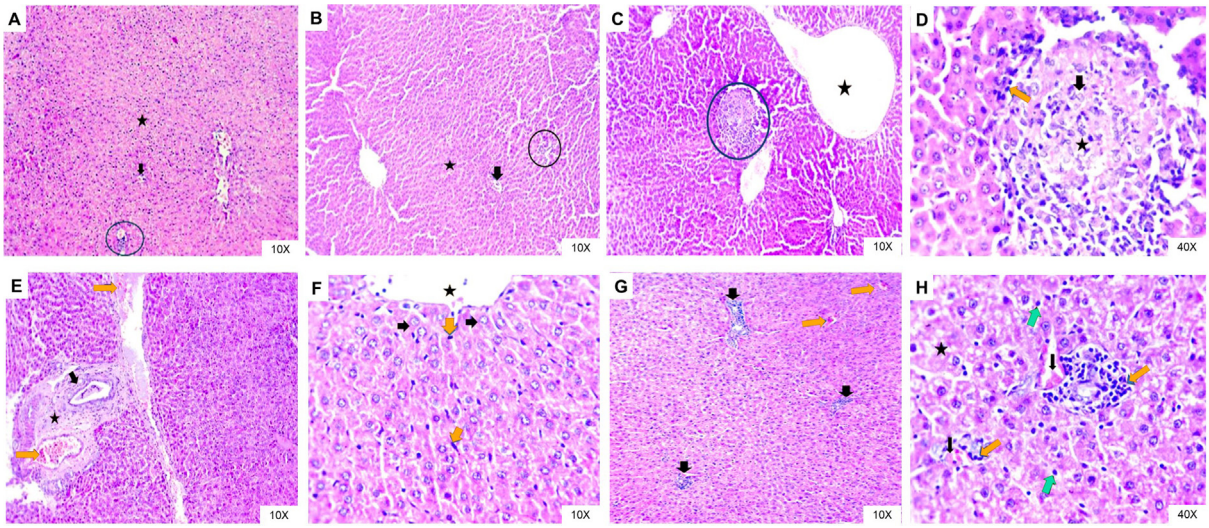

Fig. 3. Photomicrographs of liver sections in the different experimental groups stained with hematoxylin–eosin. **A, B** Control and vehicle groups show normal hepatic architectures including normal lobules, central vein (arow), sinusoids, hepatic cords (star), portal triad (circle) and Kupffer cells. **C** H_3BO_3 group shows marked widening of blood vessels (star) besides large focal necrotic area (circle). **D** High power of the C section shows necrotic hepatic parenchyma replaced with chronic inflammatory reaction (star) including epithelioid histiocyte (black arrow) admixed with chronic round cells (orange arrow) and in **E** stained section for same group shows marked fibrotic peribiliary portal triad (star), widening of congested blood vessels (orange arrows) besides proliferated bile ducts with newly formation ductulus (black arrow). **F** MEL group shows normal central vein (star) and hepatocytes (black arrows), sinusoids with prominent active Kupffer cells (orange arrows). **G** H_3BO_3 + MEL group shows nearly normal hepatocytes with still inflammatory cells infiltrations in the portal areas (black arrows), besides mild congested blood vessels (orange arrows) and prominent Kupffer cells. **H** High power of the previous section shows degenerated hepatocytes (star) with mild round inflammatory cells infiltrations in the portal triad (orange arrows) besides mild congested portal blood vessels (black arrows) and prominent Kupffer cells (green arrows).

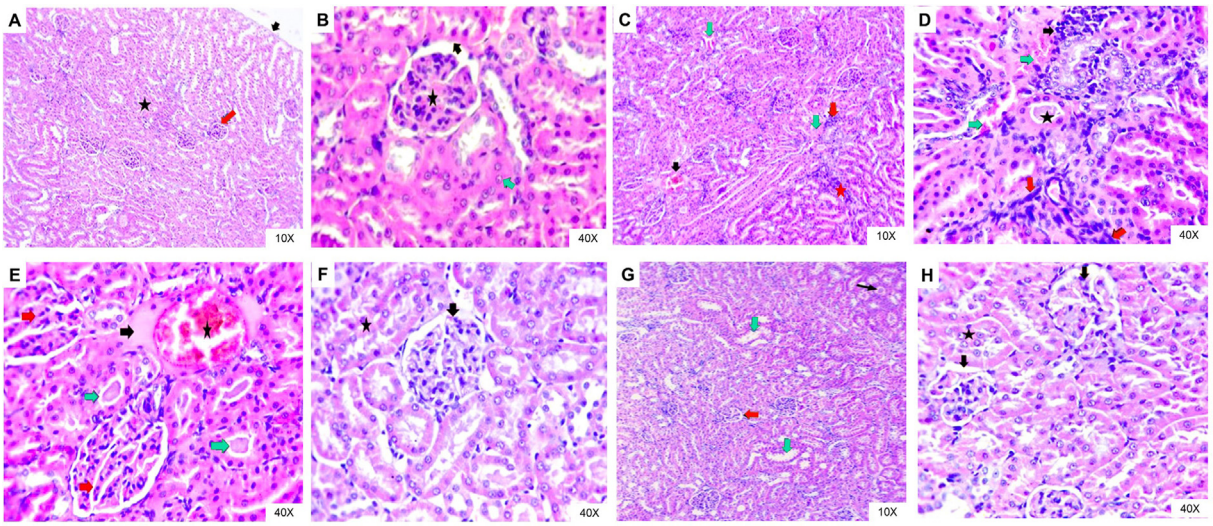

Fig. 4. Photomicrographs of kidney sections in the different experimental groups stained with hematoxylin–eosin. **A** Control group shows normal renal cortex structures including renal capsule (black arrow), glomeruli (red arrow) and renal tubules (star). **B** Vehicle group shows normal glomerular tufts (star), glomerular space and its capsule (black arrow) followed with normal proximal renal tubules epithelium and interstitial tissue structures (green arrow). **C** H_3BO_3 group shows multifocal interstitial hemorrhages (green arrows), congested blood vessels (black arrow) interstitial round cells (red arrow) and\or fibroblast proliferation (star). **D** High power of the C section shows extravasated intact erythrocytes (green arrows), increase interstitial cellularity with chronic inflammatory reaction including active fibroblasts (red arrows) and\or chronic round cells (black arrow) besides injured renal epithelium, which sometimes contains granular casts (star).**E** High power of the C section shows engorged blood vessels (star) surrounded by edema (black arrow) followed by injured renal tubules, which contain casts (green arrows), besides lobulated glomerular tufts (red arrows). **F** MEL group shows normal glomeruli (arrow) and proximal renal tubules (star). **G** H_3BO_3 + MEL group shows nearly normal renal parenchyma with cystic dilatation of many renal tubules (green arrows) and a few granular casts (black arrow) with still lobulated a few glomeruli (red arrow). **H** High power of the G section shows nearly normal renal tubules epithelium (star) and still lobulated glomeruli (arrows).

materials with collagen fibres and fibroblasts), followed by abnormal compact bone structures represented by more prominent congested blood vessels and widening of Haversian canal surrounded with waving bone (polymorphic osteocytes within narrowing lacunae among unarranged multi-stained Haversian system), active osteoblasts in the endosteum layer followed with nearly normal bone marrow. Other sections showed more dense fibrous connective tissue in the periosteum with osteoclast extended to degenerated compacted bone with prominent widening of the Haversian system, which contains fibrosis and/or osteoplastic reactivity besides congested blood vessels besides more reactive bony cellularity (Fig. 5C-F). Regarding sections from the bone of the group received H_3BO_3 + MEL revealed restoration of the normal bony

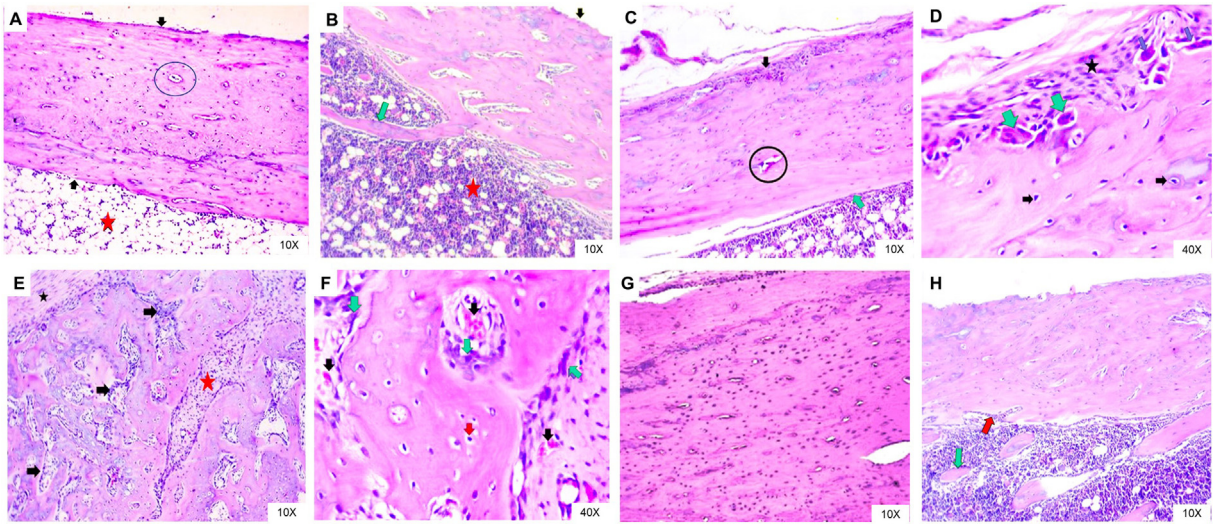

Fig. 5. Photomicrographs of bone sections in the different experimental groups stained with hematoxylin–eosin. **A** Control group shows normal compact bone as an osteocyte inside lacunae and blood vessels inside Haversian canal (circle) among homogenous matrix within normal periosteum and endosteum (black arrows) followed by normal bone marrow structure (star). **B** Vehicle group showing normal compact bone as osteocyte cells inside lacunae and blood vessels inside widening Haversian canal among homogenous matrix within normal periosteum (black arrow) and endosteum followed by active bone marrow structure (star) between bone trabeculae (green arrow). **C** H_3BO_3 group shows osteoclastic reactivity in the periosteum layer (black arrow), followed with abnormal compact bone structures represented with more prominent congested blood vessels and widening of Haversian canal surrounded with waving bone (circle), active osteoblasts in the endosteum layer (green arrow) followed with nearly normal bone marrow. **D** High power of the C section shows increase cellularity in the periosteum including active osteoclastic (green arrows) admixed with epithelized histiocytes and some debris materials with collagen fibers and fibroblasts (star), followed with polymorphic osteocytes within narrowing lacunae among unarranged multi-stained Haversian system (black arrows) and in **E** stained section for same group shows more dense fibrous connective tissue in periosteum (black star) with osteoclast extended to degenerated compacted bone with prominent widening of Haversian system which contain fibrosis and\or osteoplastic reactivity (black arrows), besides congested blood vessels besides more reactive bony cellularity (red star). **F** High power of the E section shows mature fibrous connective depositions and very wide Haversian canal which have congested blood vessels (black arrows) and active osteoblasts (green arrows), pointed osteocytes inside wide lacunae (red arrow). **G** MEL group shows normal compact bone structures. **H** H_3BO_3 + MEL group shows restoration the normal bony structures with still a focal wave bone and reactive osteoblasts (red arrow) and narrowing trabeculae (green arrow).

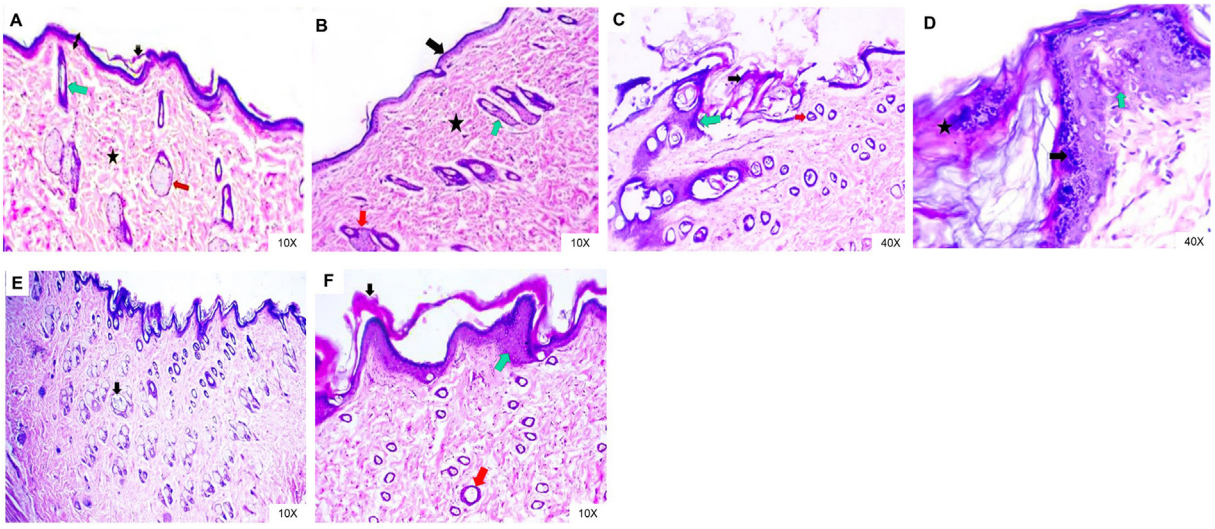

Fig. 6. Photomicrographs of skin sections in the different experimental groups stained with hematoxylin–eosin. **A, B** Control and vehicle groups show normal epidermis with non-living keratin layer (black arrow) followed with stratified squamous epithelium (double heads arrow), and dermis with normal supporting adnexal structures including sebaceous glands (red arrow), hair follicles (green arrow) and blood vessels among fibrous tissues (star). **C** H_3BO_3 group shows prominent acanthosis (green arrow) and hyperkeratosis (black arrow) and multi spaces as a remnant of destructed hair follicles (red arrow), besides abundant and cystic glands among dissociation fibrous tissues. **D** High power of the C section shows prominent granular layer cells in the epidermis (black arrow) with abundant hyperkeratosis (star) and found some epithelial cells drooped in the epidermis due to destructed basal layer admixed with spindle cells among dissociated collagen fibers (green arrow). **E** MEL group shows normal epidermal and dermal structures with abundant adnexal glands and hair follicles (arrow). **F** H_3BO_3 + MEL group still shows mild acanthosis (green arrow) and hyperkeratosis (black arrow) and focal dermis hypercellularity besides still cystic hair follicles (red arrow).

structures with still a focal wave bone and reactive osteoblasts and narrowing trabeculae (Fig. 5H).

The examined skin sections from control (Fig. 6A), vehicle (Fig. 6B) and MEL (Fig. 6E) groups revealed normal epidermis with non-living keratin layer followed by stratified squamous epithelium layers and dermis with normal supporting adnexal structures including sebaceous glands, hair follicles and blood vessels among fibrous tissues. While skin sections from H_3BO_3 rats group showed prominent multifocal acanthotic epidermal layers (acanthosis and hyperkeratosis) and multi-spaces as a remnant of destructed hair

follicles besides abundant and cystic glands among dissociation fibrous tissues (Fig. 6C). Other section showed prominent granular layer cells in the epidermis with abundant hyperkeratosis and some epithelial cells drooped in the epidermis due to a destructed basal layer admixed with spindle cells among dissociated collagen fibers (Fig. 6D). Concerning the skin section of the H_3BO_3 + MEL group, the examination came back to normal epidermal and dermal structures with abundant adnexal glands. Other sections still showed mild-to-moderate acanthosis and hyperkeratosis besides abnormal hair follicles and cystic glands with or without focal dermis hypercellularity (Fig. 6F).

Overall, no histopathological alterations were observed in the liver, kidneys, bone and skin in the vehicle and MEL groups, different degrees of histopathological changes and distribution were observed in these organs in the H_3BO_3 group, while the decreasing in the severity and distribution of the histological lesions were observed in the H_3BO_3 + MEL group in comparison to the H_3BO_3 group (Table 4).

4. Disscussion

Dramatic augmentation in exposure to toxic chemicals beyond the minimum permissible exposure in the last decades has induced harmful health effects and threatened the life of living organisms. Thus, different strategies that adjust the toxicant effects on physiological processes are essential to public health (Akefe et al., 2017). In recent years, melatonin protective effects against occurrence and development of organs damage caused by toxic agents have been well studied (Wang et al., 2018).

The impacts of exposure to different toxicants are specifically related to the liver, the fundamental organ of detoxification and kidneys the main organ of xenobiotics excretion (Gupta, 2015). ALT and AST enzymes mainly inspected to evaluate hepatocellular damage, while GGT is a sensitive index of the presence of hepatobiliary disease. This study revealed that rats exposed to H_3BO_3 showed a significant reduction in the activity of serum ALT associated with a non-significant change in serum AST activity (Table 1) and this may be due to decreased hepatocellular production or release and/or inhibition of enzymes activity that was occasionally observed in toxicological conditions (Gad, 2016; Kurtz and Travlos, 2018). However, increased serum GGT activity in the rats group exposed to H_3BO_3 may be secondary to the hepatobiliary toxicity condition (Haschek et al., 2013).

Alkaline phosphatase is an important enzyme for the plasma membrane and endoplasmic reticulum of the tissues being studied, which is expressed in various body tissues (Editore, 2014). The current results revealed a significant decline in serum ALP activity in the group exposed to H_3BO_3 and this may be related to the sensitivity of ALP enzyme to metal toxicity, which may inhibit the enzyme activity and change the manner of its isoenzymes (Mercurio, 2017). Boron is a non-metal, but it exhibits some metallic properties (Cranwell and Page, 2021), since it is a member of the metalloids (Mercurio, 2017).

The assessment of serum creatinine and urea concentrations indicates renal diseases. According to the results, the exposure of animals to H_3BO_3 induced a marked increase in serum creatinine concentration, which may reflect the renal dysfunction condition (Barger and MacNeill, 2015). However, a significant decline in

Table 4
Semi-quantitative evaluation of the histopathological alterations after 28 days of starting the experiment in different rat groups.

Lesions criteria	Control	Animal groups			
		Vehicle	H_3BO_3	MEL	H_3BO_3 + MEL
-Liver					
Inflammation (hepatitis)	–	–	+3	–	+2
Interstitial necrosis	–	–	+3	–	+/–
Hemorrhages/congestion	–	–	+3	–	+1
Blood vessels dilatation	–	–	+2	+/–	+1
Kupffer cells hyperplasia	–	–	+1	+/–	+3
Perivascular edema	–	–	–	–	+/–
Degenerations	–	–	+2	–	+1
Widening of sinusoids	–	–	+2	+/–	+1
Fibrotic trade and cholangitis	–	–	+1	–	–
-Kidneys					
Renal blood vessels congestion	–	–	+2	–	+1
Interstitial hemorrhage	–	–	+1	–	+/–
Glomerular and tubule necrosis	–	–	+2	–	+1
Tubular degeneration	–	–	+3	–	+1
Hyaline/granular Castes	–	–	+3	–	+2
Cystic dilatation	–	–	+2	–	+1
Nephritis (interstitial)	–	–	+3	–	+1
Shrinking tufted	–	–	+3	–	+1
Interstitial edema		–	+1	–	+/–
-Bone					
Hypercellularity of periosteum	–	–	+3	–	+1
Waving bone	–	–	+2	+1	+3
Widening of Haversian system	–	–	+3	+1	+1
Congested blood vessels	–	–	+3	–	+1
Active osteoblasts	–	–	+3	–	+1
Active osteoclast	–	–	+3	–	+1
Pyknotic osteocytes	–	–	+3	–	+1
Thinning bone trabeculae in bone marrow	–	–	+3	–	+1
Active bone marrow	–	–	+/–	+1	+3
-Skin					
Acanthosis/hyperkeratosis	–	–	+2	–	+1
Inflammatory cells infiltration	–	–	+1	–	–
Cystic glands	–	–	+2	–	+1
Destructed hair follicles	–	–	+2	–	+1
Abundant dermal adnexa	–	–	+1	–	+2

Score: (–) no change, (+/–) fine to mild, (+1) mild, (+2) moderate and (+3) sever alterations.

serum urea concentration after exposure of rats to H_3BO_3 may relate to the impairment in urea synthesis due to hepatocellular dysfunction, where the serum urea concentration reflects the balance between urea synthesis in the hepatic tissue and its elimination by the renal system (Hayes, 2008).

According to the results regarding serum proteins profile (Table 2), hypoproteinemia associated with hypoalbuminemia were observed in rats exposed to H_3BO_3 and may be due to the hepatic injury, which reduces the production of albumin (Kaneko et al., 2008).On the other hand, serum albumin is the main negative acute phase protein and its synthesis may be significantly declined during the acute phase response, where amino acid absorption declines or turn off for synthesis of other proteins in this phase (Jain et al., 2011). Moreover, the level of serum α1-globulin raised significantly after exposure of rats to H_3BO_3, perhaps indicating an inflammatory condition, which may be caused by the activation of the host inflammatory response (O'Connell et al., 2005).

Generally, boric acid can induce its adverse effects via its ability to enter to the cells and then hydrolyzes as borate anion and decreases the intracellular pH, which induces the cells inhibition followed by apoptosis (Park et al., 2005). Moreover, boron as a metalloid in the form of boric acid leads to cellular toxicity by different mechanisms, including an increase in oxidative system, DNA and membrane functions impairment or the inhibition of protein function and activities (Uluisik et al., 2018).

On the other hand, co-administration of melatonin with boric acid resulted in a significant reduction in hepatorenal injury and improvement in most of the measured parameters in comparison to the group that only received boric acid. These findings may relate to the ability of melatonin to improve the hepatic detoxification and antioxidant system, consequently reducing the apoptotic rate and the necrobiotic alterations in the hepatic tissue of rats, thus decreasing the severity of morphological alterations (Zhang et al., 2017). Also, melatonin can prevent mitochondrial and endoplasmic reticulum disruption, which plays a serious role in the nephron damage pathogenesis and its progression to renal failure (Inagi, 2009; Aouichat et al., 2021).

Histopathological results of liver and kidneys sections of H_3BO_3-exposed rats confirmed the biochemical parameters results (Figs. 3 and 4 C-E). These findings are consistent with the finding of Sabuncuoglu et al. (2006) who found that subacute exposure to boric acid caused histopathological changes in kidney tissue in a dose-dependent manner. Also, Ayranci et al. (2021) found that acute boric acid administration induced histopathological changes in the liver and kidneys in male albino rats. Also, histopathological findings have confirmed the ameliorative effect of melatonin on H_3BO_3-induced hepatorenal injury. In agreement with the present results, Othman et al. (2020) reported the beneficial effect of MEL against $AlCl_3$ toxicity and improved histopathological findings in the liver and kidney tissues of male Sprague Dawley rats.

Boron appears to influence the metabolism of different essential minerals and hormones that play an important role in maintaining bone health (Dessordi and Navarro, 2017; Ciosek et al., 2021). The observed hypercalcemia, hypophosphatemia and hypermagnesemia in H_3BO_3-exposed rats in this study might be due to that boron in H_3BO_3 may lead to the reduction in urinary calcium and magnesium excretion and increase ionized calcium levels in the plasma (Dessordi and Navarro, 2017). On the other hand, the exposure of animals to excess concentrations of boric acid may increase urinary phosphorus excretion and subsequently decrease the serum concentration of it (Eisler, 2007).

According to the presented findings, declining levels of serum parathormone hormone in rats exposed to H_3BO_3 may be due to the elevation in serum levels of calcium in animals (Babíc Leko et al., 2022). However, the increased serum calcitonin level after exposure of animals to H_3BO_3 may be because of the increased secretion of calcitonin by rapid flux of stored hormone from C-cells in response to increased serum levels of calcium and magnesium in animals (Burtis and Bruns, 2015).

The steroid hormones have a direct relation with the bone, while the boron may play an important role in bone health through the formation of sex steroid hormones (Dessordi and Navarro, 2017). The exposure of rats in this study to H_3BO_3 caused a significant increase in serum testosterone and 17-beta estradiol hormones levels. These changes may be due to the effect of boron on facilitation of the formation of activated (hydroxylated) forms of these hormones and inhibition of their rapid disintegration (Rondanelli et al., 2020).

The adverse impacts of boric acid on the bone may be related to the alterations in bone-related minerals and hormones levels that could be induce morphological and/or biochemical changes in bone (Beattie and Avenell, 1992). Also, bones show highly selective uptake of boron and a significantly long retention time, which were at least threefold higher than other tissues (Bagchi and Bagchi, 2021).On the other hand, boric acid has a strong affinity for *cis*-hydroxyl groups, which was estimated in different in vivo studies and this may explain the higher concentrations of boric acid in bone (Krieger, 2010).This potent affinity for *cis*-hydroxyl is a feature of interest for the ability of boron to form bioactive and toxic molecules (Bagchi and Bagchi, 2021).

In the presented study, concurrent administration of MEL in H_3BO_3 exposed rats significantly decreased the alterations in bone-related minerals and hormones levels compared with the group exposed to H_3BO_3 only. This may be due to the boric acid that may cause a significant elevation in hepatic metallothionein concentration in exposed rats (Klaassen and Suzuki,1991), where the metallothionein plays an important role in the chelation of both essential and toxic elements where they are sequestered, transported and excreted (Sears, 2013). On the other hand, melatonin can regulate the metallothionein proteins expression, which are involved in metals and metalloids detoxification (Babula et al., 2012), so the hypothesis could be that the induced elevation of metallothionein by the effect of MEL may reduce the H_3BO_3 levels in the body systems of rats in combined group, while increasing H_3BO_3 level in excretion-related systems and consequently reduce the adverse changes, which resulted from H_3BO_3 (Hernández-Plata et al., 2015). Also, the administration of both boric acid and melatonin showed that the melatonin administration decreases bone-injuring manifestations and this may be due to the fact that melatonin is a respectable free-radical scavenger and antioxidant, which can clear up the free radicals generated by osteoclasts and protect osteocytes from the oxidative attacks (Liu et al., 2013). Melatonin also increases levels of bone alkaline phosphatase and mineralization (Maria and Witt-Enderby, 2014). Moreover, bone tissue injuries induced by H_3BO_3 as well as the ameliorative effects of melatonin on these injuries, were proved histopathologically (Fig. 5C-F, H).

PGE2 is one of the most considerable produced prostaglandins in the body, which can be produced by almost all body cell types, especially infiltrating inflammatory cells and is considered a principal mediator of inflammation (Park et al., 2006).According to the results of the current study (Fig. 2), serum PGE2 levels significantly increased after exposure of animals to H_3BO_3.This may be due to the fact that prostaglandin production promptly rises in acute inflammatory conditions as well as significantly increasing production in damaged tissue (Ricciotti and FitzGerald, 2011). The level of serum PGE2 reduced in the group administered H_3BO_3 + MEL in comparison to the group exposed to H_3BO_3 alone and this change may be due to the ability of melatonin to reduce tissue damage during inflammatory response through several wherewithal, including direct free radical scavenging and indirectly by reducing the production of agents, which share in cellular damage and the

inflammatory mediators such as PGE2 (Reiter et al., 2000; Sangchart et al., 2021). Moreover, PGE2 is one of the major inflammatory mediators, which is involved in various skin disorders and a major factor in many skin inflammation conditions, besides many other tissues (Reilly et al., 2000). Exposure of rats in this experiment to H_3BO_3 induced harmful effects on the skin tissue, which confirmed by histopathological examination, while combining treatment of MEL with H_3BO_3 reduced these adverse impacts, which were confirmed by skin histopathology examination.

Hematological parameters assessment can possess a diagnostic value of adverse impacts of chemical compounds at high toxic doses on the blood components (Arika et al., 2016). According to the erythrogram results in this work (Table 3), the exposure of animals to H_3BO_3 induced a significant increase in erythrogram parameters (RBCs count, Hb concentration and Ht value). This change may be due to the increase in the response of cell membrane of erythrocytes to erythropoietin under the effect of boron in boric acid (Nielsen et al., 1991) as well as the increase in erythropoietin activity (Hedrich, 2004) and subsequent increase of extramedullary erythropiosis (Dieter, 1994; Aysan et al., 2011). The observed alterations in erythrogram reduced in the group administered H3BO3 + MEL in comparison to the group exposed to H3BO3 alone and these may relate to the effect of melatonin cotreatment on boric acid such as decrease its retention, while increasing its excretion as a result of increased metallothionein proteins expression (Hernández-Plata et al., 2015) and subsequent decreasing of boric acid adverse effects on erythrogram.

Regarding the leukogram, exposure of animals to H_3BO_3 induced leukopenia associated with neutropenia, lymphopenia and monocytopenia (Table 3). These observed alterations may suggest an immunotoxic effect of high concentrations of boric acid (Descotes, 2004). The leukogram in the H_3BO_3 + MEL administered group showed improvement in most values in comparison to the group administered H_3BO_3 only and this may relate to MEL, which may significantly overcome the immunotoxic status induced by boric acid and play a role in restoring the peripheral leukocyte count (Carrillo-Vico et al., 2013).

According to results of this study, administration of melatonin treatment alone did not induce any negative changes on all measured parameters and this is consistent with the results of study of Prevo et al. (2000). On the other hand, according to study results of Othman et al. (2020), melatonin did not show alterations in hepatic and renal function markers in the serum of rats.

5. Conclusion

The study findings pointed out that H_3BO_3-induced various adverse toxicities, presented as hepatorenal dysfunctions, alterations in bone-related minerals and hormones levels, PGE2 as inflammatory mediator as well as hematological indices. Also, the histopathological variations in various examined organs supported these alterations. On the other hand, the co-administration of MEL with H_3BO_3 partially mitigated the H_3BO_3-induced toxicity and caused a restoration of most morpho-functional state of different organs. Collectively, these results showed the ameliorative effects of melatonin against H_3BO_3 impacts, so I can say that melatonin might represent a novel approach in counteracting the toxicity of boron and its compounds.

Author contribution

HTHI designed the study, carried out the experiment, wrote, reviewed and edited the manuscript for submission.

Funding

Not applicable.

Ethical approval

The experimental protocol was approved by Zagazig University Institutional Animal Care and Use Committee “ZU-IACUC”, Egypt (Approval No: ZU IACUC/2/F/134/2021).

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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