

Research Article

Effect of ethidium bromide on hematological, biochemical, and immunological parameters in mice

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Abstract

This study aimed to investigate the effect of Ethidium bromide (EB) exposure on hematological, biochemical and immunological parameters of mice. A total of 60 BALB/c female mice were acclimated and divided into 6 groups, including: a healthy control group (GC) and 5 experimental groups given orally different doses of EB; GA (3µl), GB (9µl), GD (15µl), GE (21µl) and GF (30µl) for 1 month. Based on the results, MON was decreased significantly in all experimental groups; while, tRBCs, Hb, HCT, LYM, BAS, PLT, RDW and MPV reduced significantly in GD, GE and GF. Significant increases were observed in values of MCV, MCH, tWBCs and NEU, particularly in GD, GE and GF groups. However, no significant change was detected in MCHC and EOS. In biochemical factors, the HDL and MIP-1α were decreased significantly in GB, GD, GE and GF; whereas, increases in ALT, ALP, MDA, BUN, BIL and LDH were found significantly in GD, GE and GF groups. Also, AST, Cr and LDL were raised significantly in all experimental groups. For GLU, significant increases were recorded in GB and GD, while significant decreases in GF. For immunological parameters, the IgG was increased significantly in all experimental groups; whereas, a significant reduction in IgM was detected in GD. This study showed that the severe EB poisoning had obvious abnormalities in hematological, biochemical, and immunological parameters in mice. The identification of powerful prognostic markers is important for the management of patients with EB poisoning in emergency settings; therefore, further studies are required to clarify the mechanisms of toxicity and the adverse role of EB on tissues and organs.

Keywords: Toxic effect, Erythrocyte, Antioxidant, Lipid profile.

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Introduction

Nearly every activity leaves some kind of waste in the environment. The risk posed to human health by any untested anthropogenic chemicals in our environments (Sharholly et al. 2008; Bushnell et al. 2010). There are three basic exposure pathways: inhalation, ingestion, and skin contact (Prata et al. 2020). Although the human body can tolerate certain amounts of chemicals, it naturally attempts to eliminate substances that are harmful or not used throughout either kidney, filters substances out of the blood and excretes them in urine or liver that

detoxifies and converts chemicals to less toxic ones to remove it in feces, sweat and inhalation (Pizzorno 2015; Sarwar et al. 2017; Limaye et al. 2018). However, people respond to chemical exposure in different ways since some people may be exposed to a chemical and not get sick; while others may be more sensitive to chemicals and get sick more rapidly or have more severe reactions (Kelly and Fussell, 2012; Vandenberg et al., 2012).

As a tricyclic aromatic, heterocyclic compounds known as phenanthridine were first discovered by Swiss chemists Amé Pictet and H.J. Ankersmit in

1891. From 1930 to 1952, many phenanthridine derivatives were synthesized and tested, and some possessed highly effective pharmacological effects against the major protozoan parasites of cattle, *Trypanosoma vivax* and *T. congolense* (Fymat 2017; Ray & Dhara, 2021). In 1952, a modified compound was synthesized by replacing the methyl group with the ethyl group, which was more productive, stable, and less toxic. The original compound was ethidium bromide (EB) (Roy Chowdhury et al. 2010; Lalchandama 2016). Soon after the discovery of the DNA structure and function with the development of molecular techniques, numerous studies have demonstrated the role of EB in the rapid inhibition of DNA synthesis through the formation of a planar ring system between base pairs of the DNA (Amirijavid & Mohammadi 2014; Zhao et al. 2018; Del Giudice & Wolf 2021). In the last decades, it was revealed that EB is responsible for helix distortion of free minicircles and inhibiting replication initiation, and accumulated effects of these activities lead to DNA loss and ultimate cell death (Kahn & Crothers 1993; Roy Chowdhury et al. 2010; Fogg et al. 2021).

EB is a household name in biology and arguably the most popular stain in molecular research as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and other nucleic acid separation (Vätavu et al. 2012; Armstrong & Schulz 2015; Lalchandama 2016). EB is described as a dark red, crystalline, non-volatile solid, moderately soluble in water, which fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light (Khosravi et al. 2017; Wang et al. 2020). After an acute exposure, moderate toxicity due to direct frequent handling and irritation to the upper respiratory tract, mouth, eyes, and skin may occur (Geerling et al., 2001; Saeidnia and Abdollahi, 2013). In Iraq, there was only one report describing the insecure access and lack of safety for disposal procedures of EB in the laboratory and molecular biology (Al-Hussaini 2016). Therefore, this study aimed to investigate the toxic effects of EB on mice's hematological, biochemical, and immunological

parameters.

Materials and methods

Ethics: The current study was licensed by the Scientific Committee of the College of Education for Pure Sciences, Wasit University, Wasit Iraq.

Study animals: A total of 60 BALB/c female mice, aged ≤ 4 months and weighted 26.3-32.9g, were obtained from the private animal house in Baghdad Province, Iraq. All animals were acclimated for 72 hours, fed the pellets, drunk filtered tap water, and exposed to 12/12 hours of light and dark conditions. Randomly, mice were divided equally into six groups (A-F) as follows: (1) Group A (GA), mice were given a daily oral dose 3 μ l of EB (10mg/ml) for 30 days, (2) Group B (GB), mice were given a daily once oral dose 9 μ l of EB (10 mg/ml) for 30 days, (3) Group C (GC), mice were considered as healthy control, and not exposed to EB, (4) Group D (GD), mice were given a daily once oral dose 15 μ l of EB (10 mg/ml) for 30 days, (5), Group E (GE), mice were given a daily once oral dose 21 μ l of EB (10 mg/ml) for 30 days and (6) Group F (GF), mice were given a daily once oral dose 30 μ l of EB (10 mg/ml) for 30 days. During the experiment, mice were continued with similar feeding, drinking and photoperiod conditions.

Collection of samples: Under general anesthesia [xylazine (10mg/kg) and ketamine (100mg/kg) / IP], a terminal procedure was applied to collect blood directly through the cardiac puncture into an anticoagulant EDTA vacutainer tube using a disposable syringe (20 Gauge). All blood samples were tested by the fully automated Mythic 18 Vet haematology analyser to detect the blood parameters, viz. total red blood cells (tRBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (tWBCs), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS), RBC distribution width (RDW), platelets (PLT), and mean

platelets volume (MPV). Post hematology, the tubes of blood were centrifuged (4000rpm/ 15min) to collect sera that were kept frozen into labeled Eppendorf tubes until be used for immunology and biochemical tests.

Biochemical and immunological testing:

Following the manufacturers' instruction of sandwich enzyme-linked immunosorbent assay (ELISA) kits (SubLong Biotech, China), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin (BIL), blood urea nitrogen (BUN), creatinine (Cr), glucose (GLU), high and low-density lipoprotein cholesterol (HDL and LDL, respectively), immunoglobulin G (IgG), macrophage inflammatory protein 1-alpha (MIP-1 α), and lactate dehydrogenase (LDH) were measured at 450nm using the microplate ELISA reader (BioTek, USA). The concentrations of parameters were estimated using the standard curve throughout plotting the standard ODs in Y-axis, and the respective concentrations in X-axis with interpolating the ODs of sera and plasma to evaluate their concentration.

Statistical analysis: The data were documented in an excel sheet using Microsoft Office Excel (version 2016) and analyzed using GraphPad Prism (version 6.01). Analysis of variance (ANOVA) was used to detect significant differences between the values (Mean \pm Standard Error) of the six study groups at a probability (P) of <0.05 (*), <0.01 (**), <0.001 (***), and <0.0001 (****).

Results

Hematology: Significant differences ($P<0.05$) were found in groups treated with EB (Fig. 1). The tRBCs ($\times 10^6/\mu\text{l}$) showed a significant decrease ($P<0.0001$) in GD (4.1 \pm 0.14), GE (3.55 \pm 0.18), and GF (3.26 \pm 0.15) but not in GA (5.34 \pm 0.2) and GB (5.28 \pm 0.19) in comparison with the GC (5.54 \pm 0.17). A significant reduction ($P<0.0001$) in Hb (g/dl) was found in GE (10.48 \pm 0.21) and GF (9.79 \pm 0.23); however, insignificant change ($P>0.05$) was observed in GA (12.39 \pm 0.33), GB (12.27 \pm 0.43) and

GD (11.85 \pm 0.38). For HCT (%), gradual significant decreases ($P<0.0001$) were detected in GA (39.99 \pm 0.53), GB (38.88 \pm 1.31), GD (37.55 \pm 0.42), GE (36.5 \pm 0.42) and GF (35.07 \pm 0.56) when compared to GC (42.1 \pm 0.67). Significantly ($P<0.0001$), higher values of MCV (fl) were found in mice of GD (92.49 \pm 3.44), GE (105.19 \pm 4.48) and GF (109.19 \pm 4.48) in comparison to GA (75.97 \pm 3.24), GB (74.23 \pm 3.08) and GC (76.71 \pm 2.73). Although insignificant variation ($P>0.05$) was recorded in MCH (pg) in GA (23.57 \pm 1.28) and GB (23.53 \pm 1.21), a significant increase ($P<0.0002$) was reported in GD (29.42 \pm 1.9), GE (30.16 \pm 1.49) and GF (30.56 \pm 1.62) compared to GC (23.52 \pm 1.22). No significant differences ($P<0.12$) were found in MCHC (g/dl) of all experiment groups; GA (30.98 \pm 0.79), GB (32.25 \pm 2.37), GD (31.57 \pm 0.94), GE (28.71 \pm 0.55) and GF (27.97 \pm 0.79) compared to GC (30.62 \pm 1). Concerning tWBCs ($\times 10^3/\mu\text{l}$), there were significant increases ($P<0.0001$) in GB (4.95 \pm 0.16), GD (5.26 \pm 0.15), GE (5.58 \pm 0.18) and GF (5.84 \pm 0.23) but not ($P<0.0569$) in GA (4.48 \pm 0.14) compared to GC (4.08 \pm 0.14).

For LYM (%), GB (62.97 \pm 2.44), GD (61.71 \pm 2.81), GE (59.59 \pm 2.16), and GF (55.24 \pm 1.77) were reduced significantly ($P<0.0007$); while no significant difference ($P<0.328$) was found in GA (66.91 \pm 2.62) compared to GC (70.43 \pm 2.32). The MON (%) of all experiment groups; GA (1.96 \pm 0.22), GB (1.82 \pm 0.2), GD (1.96 \pm 0.2), GE (1.98 \pm 0.22) and GF (1.69 \pm 0.26) were decreased significantly ($P<0.0313$) compared to GC (2.71 \pm 0.19). For NEU (%), significant rises ($P<0.0001$) was detected in GB (34.17 \pm 1.81), GD (35.65 \pm 2.13), GE (37.56 \pm 1.49) and GF (40.46 \pm 1.45) but not ($P<0.2181$) in value of GA (31.46 \pm 2.02) compared to GC (28.37 \pm 1.34). Significantly, no differences ($P<0.38$) were observed in EOS (%) of GA (0.79 \pm 0.1), GB (0.84 \pm 0.06), GD (0.95 \pm 0.09), GE (0.93 \pm 0.08), and GF (1.06 \pm 0.07) groups compared to GC (0.83 \pm 0.14). Significant decreases

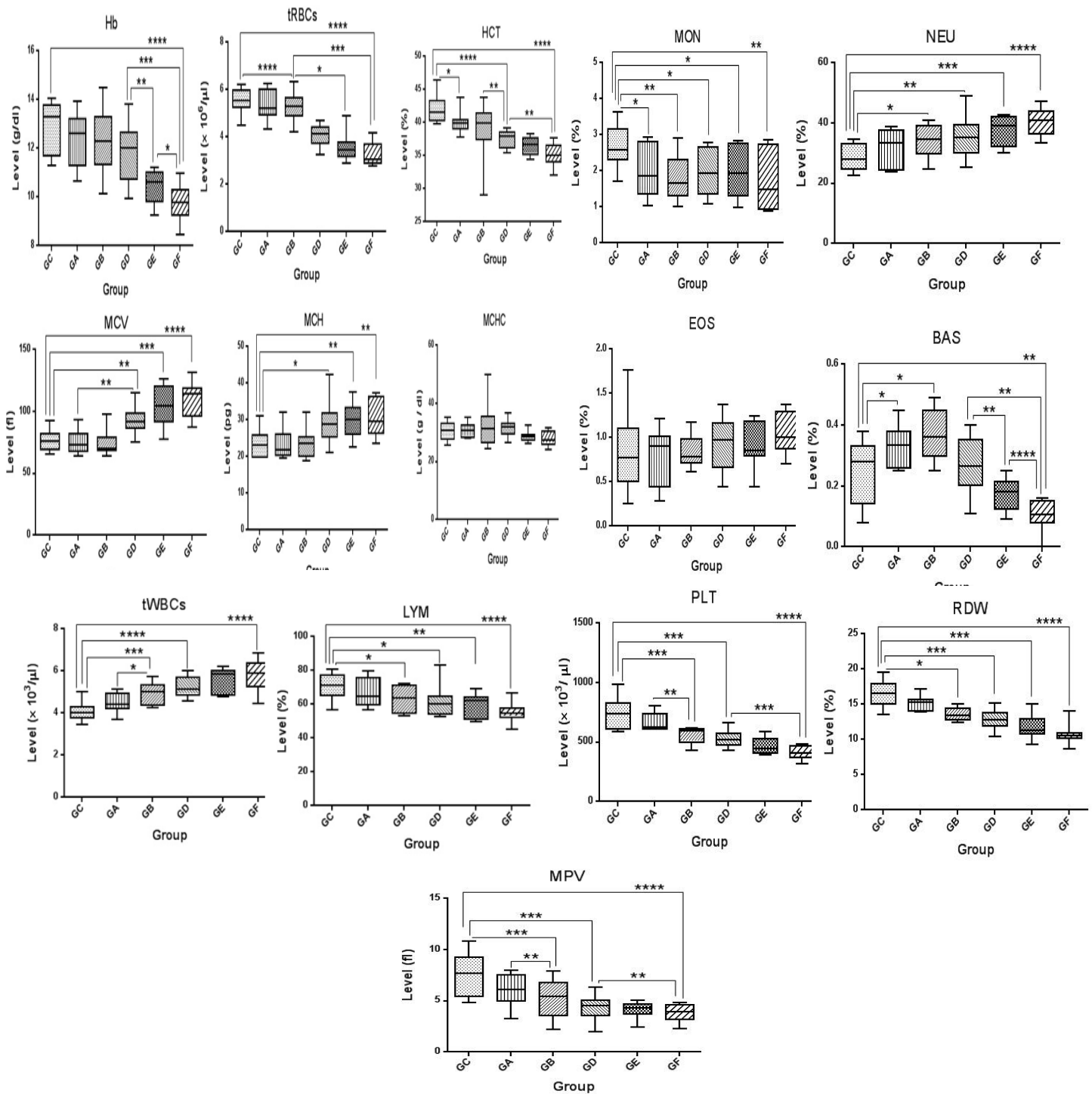


Fig.1. Values of hematology in mice of the six study groups.

($P < 0.0001$) in BAS were detected in GA (0.33 ± 0.02), GB (0.37 ± 0.03) and GF (0.1 ± 0.02); but, not in GD (0.27 ± 0.03) and GE (0.17 ± 0.02) compared to GC (0.25 ± 0.03). Regarding PLT ($\times 10^3/\mu\text{l}$), significant decreases ($P < 0.0001$) were observed in GB (556.97 ± 21.58), GD (527.67 ± 22.22), GE

(469.69 ± 21.73), and GF (411.36 ± 17.33); however, insignificant variation ($P < 0.1161$) was shown in GA (665 ± 23.35) compared to control healthy group (742.31 ± 40.59) group. For RDW (%), no significant difference ($P < 0.1025$) appeared in GA (15.1 ± 0.32); however, there were significant decreases

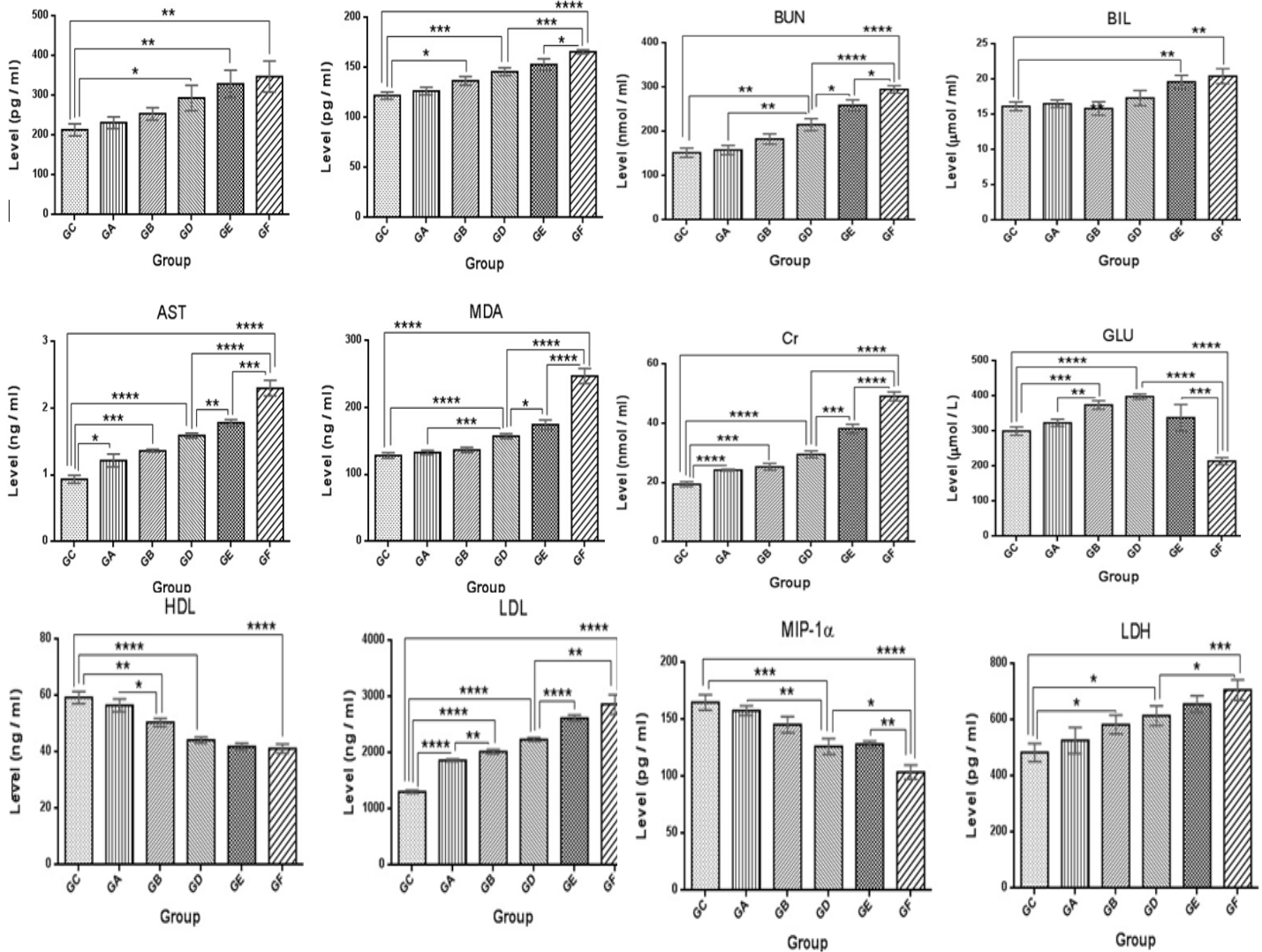


Fig.2. Values of biochemical markers in mice of the six study groups.

($P < 0.0001$) in GB (13.56 ± 0.28), GD (12.87 ± 0.48), GE (11.85 ± 0.57), and GF (10.7 ± 0.43) in comparison to GC (16.5 ± 0.59). Significantly, the highest decreases ($P < 0.0001$) in MPV (fl) were observed in GE (4.13 ± 0.25) and GF (3.83 ± 0.25) while the lowest ($P < 0.0003$) in GB (5.29 ± 0.6) and GD (4.29 ± 0.4), but not ($P < 0.0504$) in GA (6.13 ± 0.46) compared to GC (7.5 ± 0.65).

Biochemical parameters: The findings of this study revealed that there were significant differences ($P < 0.05$) in the biochemical and immunological parameters of experimentally treated groups compared to the control one (Fig. 2). Regarding the ALT (ng/ml), although significant increases ($P < 0.01$)

were reported in GD (268 ± 41.33), GE (329.5 ± 34.46), and GF (347.8 ± 38.9), no significant variation ($P > 0.05$) was observed in GA (231.8 ± 14.82) and GB (254.3 ± 15.63) compared to GC (213.9 ± 14.99). For ALP (pg/ml), no significant difference ($P < 0.3902$) was detected in GA (126.6 ± 3.77); however, a significant increase ($P < 0.0001$) was seen in GB (136.8 ± 4.33), GD (145.9 ± 3.84), GE (153.1 ± 5.57) and GF (165.8 ± 2.12) compared to GC (122 ± 3.61). Significantly, there were increases ($P < 0.0001$) in AST (ng/ml) in GA (1.22 ± 0.09), GB (1.36 ± 0.02), GD (1.59 ± 0.04), GE (1.78 ± 0.05) and GF (2.3 ± 0.12) in comparison with GC (0.93 ± 0.06). Elevation in MDA (ng/ml) was

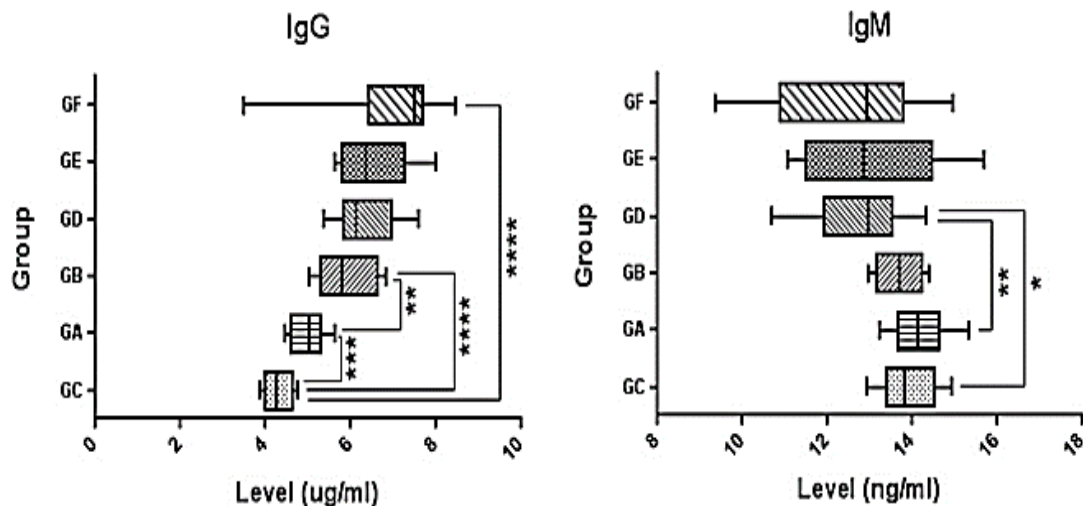


Fig.3. Values of immunological markers in mice of the six study groups.

significant ($P < 0.0001$) in GD (157.15 ± 3.68), GE (174.51 ± 6.99) and GF (247.17 ± 11.11), and insignificant ($P > 0.05$) in GA (132.92 ± 3.46) and GB (136.64 ± 3.86) compared to GC (128.25 ± 4.07).

The concentration of BUN (nmol/ml) was elevated significantly ($P < 0.0001$) among animals of GD (215.7 ± 13.47), GE (259.1 ± 12.65) and GF (295.3 ± 8.17) but not ($P > 0.05$) in GA (158 ± 10.51) and GB (183.2 ± 11.32) compared to GC (152.3 ± 10.4). For BIL ($\mu\text{mol/L}$), GA (16.54 ± 0.53), GB (15.87 ± 0.96) and GD (17.34 ± 1.08) were differed insignificantly ($P > 0.05$) compared to GC (16.17 ± 0.62); however, there were significant increases ($P < 0.0013$) in GE (19.63 ± 0.94) and GF (20.45 ± 1.06). Concerning the level of Cr (nmol/ml), gradual increases were significant ($P < 0.0001$) in GA (24.23 ± 0.32), GB (25.33 ± 1.12), GD (29.51 ± 1.15), GE (38.22 ± 1.49) and GF (49.14 ± 1.52) compared to GC (19.47 ± 0.84). Levels of GLU ($\mu\text{mol/L}$) were differed insignificantly ($P > 0.05$) in GA (323.7 ± 10.37) and GE (338.1 ± 37.69), increased significantly ($P < 0.0001$) in GB (374.8 ± 12) and GD (398.3 ± 7.5), and decreased significantly in GF (214.6 ± 9.85) compared to GC (300.2 ± 12.23). For HDL (ng/ml), decreases were shown significantly ($P < 0.0001$) in GB (50.49 ± 1.46), GD (44.21 ± 1.08), GE (41.97 ± 1.12) and GF (41.22 ± 1.6), and

insignificantly ($P < 0.4018$) in GA (56.56 ± 2.29) compared to GC (59.27 ± 2.18). Whereas in LDL (ng/ml), significant increases ($P < 0.0001$) was observed in all experimental animals of GA (1868.2 ± 23.12), GB (2022 ± 38.96), GD (2238.6 ± 32.56), GE (2619.1 ± 54.66), and GF (2863.6 ± 173.88) compared to control animals (1307.8 ± 26.45). For MIP-1 α (pg/ml), values were decreased significantly ($P < 0.0001$) in GD (126.4 ± 7.03), GE (128.3 ± 2.83) and GF (103.8 ± 6.26), and insignificantly ($P > 0.05$) in GA (157.9 ± 4.13) and GB (145.5 ± 7) compared to GC (165 ± 6.69). The LDH (pg/ml) was increased significantly ($P < 0.0006$) in GB (583.5 ± 33.26), GD (615.3 ± 34.91), GE (656.4 ± 29.73) and GF (706.8 ± 36.15), and insignificantly ($P < 0.4586$) in GA (527.2 ± 46.62) compared to GC (484.2 ± 32.41).

Immunological parameters: The findings of immunological parameters were showed significant differences ($P < 0.05$) between the treated groups and the control group (Fig. 3). Regarding IgG (ug/ml), gradual increases were significant ($P < 0.0001$) in GA (4.99 ± 0.13), GB (5.87 ± 0.21), GD (6.28 ± 0.22), GE (6.55 ± 0.26) and GF (6.99 ± 0.44) compared to GC (4.28 ± 0.1). For IgM (ng/ml), no significant variation ($P > 0.05$) was observed in GA (14.15 ± 0.2), GB (13.69 ± 0.16), GE (13.07 ± 0.49) and GF (12.44 ± 0.57); however, there was a significant decrease ($P < 0.0083$)

in GD (12.72 ± 0.37) compared to GC (13.92 ± 0.21).

Discussion

Our findings revealed that exposure to different doses of EB could have variable hematological effects. Our results revealed a significant decrease in values of MON among all experimental group, while tRBCs, Hb, HCT, LYM, BAS, PLT, RDW and MPV were reduced significantly in GD, GE and GF groups. Piomelli (1981) summarized that exposure to toxic chemicals may result in alterations of RBCs functions; and in certain cases, the toxic effect requires a genetic predisposition and thus affects only a restricted number of individuals; or in other instances, the toxic effect is exerted on the hematopoietic system of every person. Sun & Wang (1991) confirmed that EB could alter the patterns of DNase I hypersensitive of the β^A -globulin gene in chicken erythrocytes. Hejtmancik et al. (2002) mentioned that mice exposed to toxicity appeared more sensitive to the formation of Heinz bodies and the development of anemia characterized by a decrease in Hb, HCT, and tRBCs. Another possibility would be alterations in bone marrow caused by chromosomal aberrations in bone marrow cells after exposure to high doses of toxic materials (Prasad et al. 2009). The production of reactive oxygen species (ROS) could be the cause of decreasing in tRBCs that possess limited antioxidant defenses, which renders the cells more sensitive to changes in the antioxidant/pro-oxidant balance (Jasper et al. 2012). Perrone et al. (2012) confirmed that RBCs of mice exposed to toxic materials are more susceptible to hemolysis, probably, due to low pH and higher oxidative stress. These declines in tRBCs, Hb, and HCT could be caused by impairment of biosynthesis of harm in bone marrow or disruptive action of EB on the erythropoietin tissue as a result, of which the viability of the cells might be affected (Aitte & Zain 2019).

In this study, a significant decrease in LYM indicates immune system deficiency. As detected with other xenobiotics among different animals,

decreasing in LYM might be due to either direct toxic action of a xenobiotic on leucopoiesis in lymphoid organs (Okediran et al. 2017), increased lysis as a result of the presence of xenobiotic in the body (Sinha et al. 2018), or existence of cortisol secreted that shortens the life of LYM, promoting the apoptosis and reducing their proliferation (Burgos-Aceves et al. 2019). Significant lowering of MON might be related to cytotoxicity (Berntsen et al., 2018), impaired bone marrow function (Roth et al. 2020) and the existence of inflammatory response (Choudhury et al. 2021). For BAS, the significant reduction might be caused by severe allergies (Chirumbolo et al. 2018) and an overactive thyroid gland (Asmat et al., 2020).

Lowered values of PLT could be produced either as a result of decreasing PLT production due to bone marrow toxicity or increasing PLT destruction and accumulation within an enlarged spleen (Lee & Lee 2016). Also, it may be associated with internal hemorrhagic foci that can be detrimental to mice because these cells may be linked to inflammatory and phagocytic responses (Swindle et al. 2012; Boor 2017). MPV results, PLT count, and low MPV levels indicate exposure to harmful substances that cause impairment in bone marrow function and inflammatory conditions (Khairkar et al. 2016). A significant reduction in RDW confirmed the iron deficiency or microcytic anemia (Hempel & Bollard 2016). There is increasing evidence that PLT count, MPV, and RDW have a significant role in discriminating between hyper-destructive and hypo-productive, decreasing PLT (Khaleel & Ahmed 2014; Negash & Tsegaye 2016; Mali et al. 2021).

Significant increases were reported in MCV, MCH, tWBCs and NEU, particularly in GD, GE and GF groups. However, all experimental groups showed no significant variation in MCHC and EOS. Increased MCV may indicate the effects on the liver and that anemia or due to the existence of a negative association of exposure to EB with adverse effects on the spleen and lymph (Kamal & Malik 2012). The high MCH could indicate the presence of large-size

RBCs containing less Hb (Ucar et al. 2019). Larsen et al. (2016) detected that the unusual increase in the percentage of NEU might be due to certain inflammatory or infectious conditions because an essential function of these factors is to provide a primary defense. Kumar et al. (2018) reported that increasing tWBCs could rise NEU due to NEU margination and not increased bone marrow production. Jia et al. (2019) mentioned that patients with significant stress should have a higher degree of tWBCs than patients with minor stress. Tissue damage from poisoning and severe ill conditions such as kidney failure can raise NEU percentage (Banaei et al. 2008).

The high presence of EOS might be related to cytotoxicity (Berntsen et al. 2018), impaired bone marrow function (Roth et al. 2020), and the existence of inflammatory response (Choudhury et al. 2021). Biochemically, the HDL and MIP-1 α were decreased significantly in GB, GD, GE and GF groups, whereas ALT, ALP, MDA, BUN, BIL and LDH were increased significantly in GE and GF groups. Also, AST, Cr and LDL concentrations were elevated significantly among all experimental groups. For GLU, significant increases were shown in GB and GD, while significant decreases in GF. Mice exhibited higher ALT and AST activities during exposure to EB may be due to liver damage that results in releasing these intracellular enzymes and raising their concentrations in blood (Banaei et al. 2008; Al-Abedi et al. 2020). Serum ALP increases may indicate a health concern with liver or gallbladder such as liver cirrhosis, hepatitis, blockage in bile ducts and gallstones (Yap & Aw 2010; Kwo et al. 2017), overactive parathyroid glands (Anwar et al. 2018), or elevated osteoblastic activity and isoenzyme level (Nizet et al., 2020). The increase in the activity of BUN and Cr in serum may refer to the development of hepatic and renal damage (e Silva et al., 2019; Yarijani et al., 2019). The observed increased MDA supports our hypothesis that the toxic effect EB was associated with its potential capacity to cause cellular damage and release ROS.

The increases in LDH are possibly derived from the death of inflammatory cells (Chan et al. 2013) or the sloughing/destruction of tissue layers in the digestive system, particularly in the small intestine (Nayak et al. 2018). Significant differences in GLU concentrations in sera between the controlled and treated mice may be a display of stress. This study suggested that increase in GLU might be associated with the inhibition of pancreatic β -cells to the secretion of sufficient amounts of insulin (Brereton et al. 2014) or due to increasing hepatic lipid metabolism (Trauner et al. 2010). Reduction in levels of GLU at the high dose of EB (30 μ l) might be explained by the decrease in appetite (Rakshit et al. 2008), reduce the absorption of the intestine (Mathur et al., 2011), severe liver illness (Bagshaw et al. 2009), or deficiencies in certain adrenal and pituitary hormones (Maghnie et al. 2005; Zeng et al. 2007).

In this study, IgG was increased significantly in all experimental groups; whereas, significant decreases in IgM were found in GD group. For many years, traditional methods for toxicological assessment have implicated the immune system as a frequent target organ of toxic insult following chronic or sub-chronic exposure to certain chemicals. This awareness of immunotoxicology was estimated by numerous authors who provided evidence that the broad spectrum of xenobiotics alters immune responses in laboratory animals and subsequently may affect the health of exposed individuals (Baik et al. 2013; Ghosh et al. 2016; Shi et al. 2021). The presence of high levels of IgG antibodies and low levels of IgM is consistent with an active ongoing immunological response to EB exposure. Moreover, these levels of IgG antibodies in experimentally drenched mice indicate chronic exposure to EB and that immunological memory response can occur with respect to EB exposure.

Conclusion

Although EB is widely used worldwide as a fluorescent stain for gel in the molecular assay, the toxic effects of this material have not been studied

well. This study confirmed that exposure to a commonly utilized dose of EB (3 μ l) did not reveal a significant change in targeted bodily markers. Nonetheless, severe effects were appeared with increased exposure to high amounts of EB, in particular 21 and 30 μ l suggesting that these doses might result in oxidative damage to RBCs leading to anemia and a variety of tissue and biochemical changes. Nonetheless, it appears that these hematological, biochemical, and immunological alterations are complex due to the interaction of multiple factors that need to be studied abundantly and demonstrate the factors that play a potential role definitely.

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