Potential Effects of Boldine on Oxidative Stress, Apoptosis, and Inflammatory Changes Induced by the Methylprednisolone Hepatotoxicity in Male Wistar Rats

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Abstract

Background: Synthetic glucocorticoid therapeutic agent methylprednisolone (MPL), when used for an extended period of time at high dose, promotes the development of reactive oxygen species (ROS)-induced liver toxicity. This study investigated the role of boldine, a natural antioxidant with anti-apoptotic and anti-inflammatory properties, against MPL-induced hepatotoxicity in male Wistar rats.

Methods: 120 rats were divided into eight equal groups: G1 (control), G2, 3, and 4 (rats orally administered 5, 10, and 50 mg boldine/kg b.w./day; respectively, for 28 days), G5 (rats intramuscularly injected with 100 mg MPL/kg b.w. only on the last three days), G6, 7, and 8 (rats administered boldine + MPL). After the last MPL injection, rats were sacrificed at intervals of 1, 24, and 48 h.

Results: There was a significant decrease in WBCs, RBCs count, and HGB levels, as well as an increase in PLT count, ALT, AST, TG, and LDL levels, and a decrease in HDL level in serum. Oxidative stress markers levels increased at all times, and gene expression of antioxidant enzymes increased at 24h. Immunohistochemical analysis revealed that cytochrome c levels significantly increased after MPL treatment. The COMET assay revealed detectable DNA lesions. There was no immune reactivity of IL-6 expressions as an inflammatory response marker.

Conclusions: Oral administration of boldine has a modulatory protective, antioxidant, and anti-apoptotic effect against free radicals.

Keywords
methylprednisolone, hepatotoxicity, oxidative stress, DNA damage, boldine, anti-oxidant properties

Introduction

Glucocorticoids (GCs) are stress hormones produced by the adrenal gland cortex. They regulate a wide range of physiological actions (metabolic, inflammatory, cardiovascular, and behavioral processes), under control of the hypothalamic pituitary adrenal (HPA) axis.¹ Pharmacologically, GCs are among the most widely prescribed and used drugs in the world to treat a wide range of medical disorders, including autoimmune diseases, inflammatory conditions, rheumatism, gastrointestinal diseases, and chemotherapeutic and immunosuppressive regimens for the treatment of both tumors and organ transplantation.²,³

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Methylprednisolone (MPL) is a synthetic glucocorticoid with moderately potentiated glucocorticoid activity that is used in human and animal medicine to investigate the effects of steroids.\(^4\)\(^5\) It is regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Clinically, MPL is administered to suppress inflammation and as an immunosuppressive agent by entering the nucleus, altering gene expression, and inhibiting pro-inflammatory cytokine production. Furthermore, it reduces the number of circulating lymphocytes, induces cell differentiation, and stimulates apoptosis in sensitive tumor cells populations.\(^6\)

Synthetic GCs, in general, cause a variety of adverse effects associated with therapeutic effects due to the use of high doses and long-term treatment. As seen in metabolic syndrome, an excess of GCs causes alterations in lipid and glucose metabolism, as well as insulin resistance, fat accumulation, visceral obesity, increased appetite, disturbances of wound healing, an increased infection risk, hypertension, dyslipidemia, and increased cardiovascular risk.\(^7\) Furthermore, prolonged use of oral GCs has been linked to Cushing’s syndrome.\(^8\)\(^9\) Short-term MPL treatment at high doses causes hyperglycemia, tachycardia, flushing, gastrointestinal symptoms, sleep disturbance, psychotic reactions, neutrophilia, and lymphopenia.\(^6\)

Boldine (1,10-dimethoxy-2, 9-dihydroxy aporphine) is the major alkaloid found in the leaves and bark of the *Peumus boldus* Molina tree (*P. boldus*) (Monimiaceae), with antioxidant and radical scavenging properties.\(^10\) It is a slow-growing, shrubby evergreen tree, commonly known as boldo.\(^11\) *P. boldus* is widely distributed and used in Central and Southern Chile, South America, and North Africa as a medicinal plant for the European market.\(^12\) *P. boldus* is traditionally taken as an herbal tea drink after eating.\(^13\) It is used to treat a wide range of diseases and symptoms, including headaches, earaches, nasal congestion, rheumatism, sleep disturbances, and infections of the urinary and digestive systems, as well as liver and gallbladder disorders.\(^14\) The leaves of *P. boldus* contain bioactive essential oils (EO), alkaloids, and flavonoids with intriguing medicinal properties.\(^15\)

Due to strong antioxidant activity of boldine, it protects biological systems from oxidative stress-mediated injuries such as liver injury, fibrosis, hepatotoxicity,\(^16\) inflammation, tumor cell proliferation, and atherosclerosis.\(^17\) Boldo leaf aqueous extract also protects rats from liver toxicity caused by paracetamol, phenobarbital, rifampicin, and isoniazid. Boldine protects the mitochondria of rat liver from oxidative damage caused by Fe/citrate.

Structurally, boldine has two phenolic hydroxyl groups.\(^18\) This alkaloid is thought to be responsible for the majority of the boldo extract’s health-promoting and pharmacological activities.\(^19\)\(^20\) Boldine has cyto-protective, anti-atherogenic, anti-platelet, anti-tumor, anti-inflammatory, immunomodulatory, hepato-protective, and anti-pyretic properties.\(^21\)\(^22\)

The present work was aimed to study the antioxidant, anti-apoptotic, and anti-inflammatory activity of boldine against changes induced by MP hepatotoxicity in a male Wistar rat model.

**Materials and Methods**

**Experimental Animals and Chemicals**

One hundred and twenty male Wistar rats (average weight 180-200 g) were obtained from the animal house of King Saud University, Riyadh, Saudi Arabia. The rats were housed in plastic cages under controlled temperature (24 ± 2°C) and lighting (12 h light/dark cycle), as well as a relative humidity of 40–70%, and were fed a standard diet and given water *ad libitum*. All animals were handled in accordance with the recommendations of the King Saud University (KSU) Ethics committee in Riyadh, Saudi Arabia (KSU-SE-19-139), which received ethical approval on 13-02-2020.

Methylprednisolone sodium Succinate 1000 mg (Chemical Formula: C26H33NaO8) was obtained from Pfizer Manufacturing Belgium NV Company. Commercial assay kits were obtained from Cayman (Cayman Chemical Company, Ann Arbor, MI, USA), and BioVision (BioVision Incorporated 155 S. Milpitas Blvd, Milpitas, CA 95035, USA) was used for measuring antioxidant defense enzyme and oxidative stress markers. Thermo Scientific (USA) provided antibodies for immunohistochemistry (IHC) of IL-6 and cytochrome c. (Waltham, Massachusetts). The DNA extraction kit was obtained from Qiagen (Hilden, Germany). Other chemicals were of high analytical reagents or grade.

**Preparation of Boldine**

Boldine was purchased from Sigma-Aldrich Chemical Co (St Louis, MO, USA). Boldine used in treatment was prepared freshly by dissolving powder in distilled water and administered orally by gavage. The dose of boldine used in this study was according to the one in Ref. [22,23].

**Experimental Design**

The experimental animals were divided into 8 groups of 15 rats each.

The rats were then treated for 4 consecutive weeks as follows:
- **Group 1:** Rats administered with distilled H₂O (Control group)
- **Group 2:** Boldine (5 mg/kg b.w.) administered daily via oral tube for 4 weeks.
- **Group 3:** Boldine (10 mg/kg b.w.) administered daily via oral tube for 4 weeks.
- **Group 4:** Boldine (50 mg/kg b.w.) administered daily via oral tube for 4 weeks.\(^22\)\(^23\)
- **Group 5:** MPL (100 mg/kg b.w.) sodium Succinate was injected intramuscularly only on the last 3 days.\(^24\)
- **Group 6:** Boldine (5 mg/kg b.w.) + MPL administered daily via oral tube for 4 weeks, and MPL (100 mg/kg b.w.) was injected intramuscularly once a day on the last 3 days of the experiment.
- **Group 7:** Boldine (10 mg/kg b.w.) + MPL administered daily via oral tube for 4 weeks, and MPL (100 mg/kg b.w.) was injected intramuscularly once a day on the last 3 days of the experiment.
Group 8: Boldine (50 mg/kg b.w.) + MPL administered daily via oral tube for 4 weeks, and MPL (100 mg/kg b.w.) was injected intramuscularly once a day on the last 3 days of the experiment.

All animals were housed in a manner that allowed free access to water and food. After the last MPL administration, five rats from each group (control and treated) were sacrificed at 1, 24, and 48 h intervals.

Sample Preparation
Immediately after decapitation of the rats, blood was collected from the trunk in an EDTA tube to measure the whole blood (hematologic measurements) and collected in a serum separator tube for biochemical analysis. The blood samples were collected in a sterile, closed plain tube and allowed to clot at 25°C. The tubes were then centrifuged at 3500 r/min for 15 min at 4°C. Serum samples were transferred to sterile Eppendorf tubes and stored at -25°C. The tubes were then centrifuged at 3500 r/min for 15 min at 4°C. Serum samples were transferred to sterile Eppendorf tubes and stored at -80°C until further analysis.

The liver was removed immediately and perfused with ice-cold saline. A portion of the liver tissue was immediately transferred to 10% buffered formaldehyde for histological and IHC examination. On the medium, a second part was weighed and stored at 4°C. Serum samples were transferred to sterile Eppendorf tubes and stored at -80°C until further analysis.

Determination of Hematological Parameters
In this study, hematologic values were measured using standard methods.

The reagents used were: TTM 5 diff diluent, TTM 5 diff Hgb lyse, TTM 5 diff fix, TTM 5 diff WBC lyse, TTM 5 diff rinse, TTM 5 diff calibrator, TTM 5 diff control plus. The parameters assessed included: red blood cells (RBC, 10⁶/μL), hemoglobin concentration (HB, g/dL), and white blood cell count (WBC, 10³/μL).

All indices were measured according to the manufacturer’s recommendations using full-automated hematology analyzer Beckman Coulter Ac T 5diff (Beckman Coulter, U.S.A.)

Biochemical Analysis
Lipid profile. The total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride concentrations in rat serum were determined using the ReflotronPlus Dry-Chemistry Analyzer (Roche, Germany). The results were expressed in mg/dl. All Reflotron strips were purchased from Roche (Germany).

Liver function tests. The concentration and activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rat serum were determined using ReflotronPlus Dry-Chemistry Analyzer (Roche, Germany).

Measurement of 11-Beta-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) enzyme activities. 11β-HSD1 enzyme activity was analyzed in liver tissues homogenates using commercial sandwich enzyme immunoassay (ELIZA) kits (Cat. NO. DR-HSD11b1-Ra, Reddotbiotech Company)

Histopathological Examination
Liver sample was fixed in 10% buffered formaldehyde for 24 h, and then washed with tap water. Dehydration was performed using serial dilutions with (methyl, ethyl, and absolute ethyl) alcohol. Specimens were cleared with xylene and then embedded in paraffin for 24 h at 56°C. The paraffin beeswax tissue blocks were cut into 5–6 μm thickness using a rotary microtome. Tissue sections were then stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Antioxidant Defense Enzyme and Oxidative Stress Biomarkers
Part of the liver was weighed and homogenized on medium according to the protocol, and it was used to determine lipid peroxidation (LPO), nitric oxide (NO), and reduced glutathione (GSH) as markers of oxidative stress, as well as glutathione reductase (GR), glutathione peroxidase (GSHPx), and superoxide dismutase (SOD) as antioxidant enzymes.

Determination of lipid peroxides (malondialdehyde levels) (MDA), nitric oxide (NOx), and reduced glutathione (GSH) levels. LPO and NOx were measured using kits from (BioVision U.S.A). GSH was measured using kits from Cayman Chemical Company, Ann Arbor, MI, USA.

Determination of Glutathione Peroxidase (GSHPx), Glutathione Reductase (GR), and Superoxide Dismutase (SOD) Activities. GSHPx, GR, and SOD were measured using commercial assay kits from Cayman Chemical Company, Ann Arbor, MI, USA.

Molecular Analysis. Total RNA was extracted from liver tissue using RNA Mini Kit (Invitrogen, USA) (Invitrogen, Thermo Fisher Scientific, PureLink™ RNA Mini Kit. Cat. No.12183018 A), following the manufacturer’s instructions. The concentration and quality of RNA were measured using Nanodrop spectrophotometer (NanoDrop, Wilmington, DE, USA) at wavelengths of 260/280 nm. Following the manufacturer’s instructions, complementary DNA (cDNA) was synthesized from RNA using a High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Cat. No. 4368814). Real-time polymerase chain reaction (PCR) analysis was performed on samples in triplicates. PCR amplification included non-template controls containing all reagents, except cDNA. Real-time PCR was performed using Power SYBR Green (Life Technologies, Carlsbad, CA) and an Applied Biosystems 7500 Instrument (Foster City, CA). The typical thermal profile was 95°C for 3 min, followed by 40 cycles of
95°C for 1 s, 61°C for 20 s, 72°C for 2 min, and termination at 4°C. After PCR amplification, the Δ Ct was calculated by subtracting β-actin Ct from each sample Ct, using the Applied Biosystems Step One Instrument software. The method of Pfaffl was used for data analysis. PCR primers for SOD, GSHPx, GR, 11b-HSD1 and 11b-HSD2 genes were synthesized by Integrated DNA Technologies, Inc, Illinois, USA.

Primers for the genes of interest were designed as follows: SOD [(F) 5'-GAG CAG AAG GCA AGC GTG GAA-3', (R) 5'-CCA CAT TGC CCA GTT CTC-3']; GPx [(F) 5'-AAC GTG GCC TCG CAA TGA-3', (R) 5'-GGG AAG GCC AGG ATT CGT AA-3']; GR [(F) 5'-TTC TGG AAC TCG TCC ACT AGG-3', (R) 5'-CCA TGT GGT TAC TGC ACT TCC-3']; 11b-HSD1 [(F) 5'-CAA TGG CAT TGT TGT CG-3', (R) 5'-GAA CCC ATC CAA AGC AA-3']. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene.

Biomarker of Cell Death

Total DNA Preparation, Extraction, and Fragmentation. Liver tissue (25 mg) was placed in a 1.5 mL microcentrifuge tube and incubated at 56°C until the tissue is lysed after which 180 μl ATL buffer and 20 μl proteins K (Qiagen, Hilden, Germany, cat #69504) were added. Total DNA was extracted using a DNeasy blood & tissue kit (Qiagen, Hilden, Germany, cat #69504) and eluted with 200 μl elution buffer, according to the manufacturer’s instructions. Extracted DNA was quantified using NanoDrop-8000 (Waltham, USA), and its integrity was evaluated using agarose gel (1.5%) electrophoresis. Gels were illuminated using 300 nm ultraviolet light and a photographic record was obtained.

Biomarker of genotoxicity (Comet assay). Genotoxicity was evaluated in blood using Comet assay kit (3-well slides) (ab238544, abcam, UK). The single cell gel electrophoresis (SCGE) assay is used to quantify and analyze DNA damage in individual blood cells. Lymphocytes were separated from whole blood and suspended in PBS at a concentration of 30 million cells/ml. The electrophoresis compact power supply was turned on for 30min at 25 V and ~300 mA (International Biotechnologies, Inc, New Haven, CT). Observations were made under a fluorescent microscope (Nikon Eclipse TI-E, Japan). A one-tailed test was used to examine the effect of dose on the length of the DNA migration. The tail intensity is measured automatically by image analysis software. The following formula was used to calculate the tail moment: tail moment = tail length x tail intensity/100.

Immunohistochemical (IHC) for Detection of Cytochrome c and IL-6

This procedure was performed on 5 μM paraffin liver sections. The sections were incubated for 12 h at 4°C in a blocking solution with cytochrome c antibody (Clone 7H8.2C1) (mouse monoclonal) for the detection of cytochrome c and with IL-6 antibody for the detection of IL-6. Re-equilibration and washing procedure with PBS was done in order to incubate the sections with Horseradish Peroxidase (HRP) antibody conjugates at concentration ratio of 1:2500. Following this, the specimens were re-washed with PBS and incubated with a .2% solution of 3,30-diaminobenzidine (DAB) until the desired stain intensity at RT was obtained. After appropriate washing in PBS, slides were counterstained with hematoxylin, dehydrated in a graded series of ethanol, and finally mounted with di-n-butyphthalate-polysterene (DPX). Three independent observers carried out blind measurements of the immunoreactivity, and the total number of positively stained cells was recorded.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences program (SPSS) software (ver.22; SPSS Inc, Chicago, IL, USA). All the P-values in this study were two-sided, and ≤0.05 was considered significant. Further comparisons among groups were made according to post-hoc LSD test. Continuous and categorical variables were displayed as means ± Standard Deviation (SD) and percentages, respectively.

Results

The present study evaluated the hematological and biochemical alterations generated by MPL in male rats compared to the control group. Furthermore, histopathological and immunohistochemical analysis were performed. The antioxidant defense enzyme activities, oxidative stress markers, and the gene expression of SOD, GPx, GR, and 11b-HSD1 in liver, were evaluated. The potential protective effect of boldine was studied. From the results, when compared to the control groups, boldine supplementation alone had no significant effect on the markers evaluated in the liver throughout all time intervals.

Biochemical Parameters

Hematological Parameters. The rats treated with MPL had significantly lower WBCs and RBCs count and hemoglobin (HGB) levels, while their blood platelet (PLT) count increased in 1, 24, and 48 h, when compared to control group (Figures 1A–D). In the boldine + MPL treatment group (BOL 5,10, 50 mg/kg+ MPL), an increase in WBCs and RBCs count, HGB levels, as well as a decrease in PLT count at 1, 24 and 48 h, compared to the MPL group were observed. The boldine supplementation provided a significant protection against the MPL-induced hematological changes, at all-time intervals.

Liver Function. The serum biochemical analysis revealed significant increase in activity levels of ALT and AST at 1 and 24
in MPL-treated groups compared to the control group (Table 1). However, the groups treated with boldine + MPL (BOL 5, 10, 50 mg/kg + MPL) showed a decrease in serum activity levels of ALT and AST in both time intervals compared to MPL group. Boldine supplementation was observed to provide a significant protection against the MPL-induced liver damage.

**Table 1.** Influence of boldine and/or methylprednisolone on total alanine aminotransferase (ALT) (u/l) and aspartate aminotransferase (AST) (u/l) in the different rat groups.

| Groups | Parameter | Control | BOL 5 mg | BOL 10 mg | BOL 20 mg | BOL 30 mg | BOL 50 mg | BOL 100 mg | BOL 5 mg + MPL 100 mg | BOL 10 mg + MPL 100 mg | BOL 50 mg + MPL 100 mg |
|--------|-----------|---------|----------|-----------|-----------|-----------|-----------|-----------|----------------------|------------------------|------------------------|
| ALT (1 h) | 86.74 ± 0.36a | 83.6 ± 1.09a | 83.28 ± 2.39a | 85.92 ± 1.39a | 106.0 ± 3.06b | 100.84 ± 1.5c | 100.48 ± 1.2c | 100.18 ± 2.2c |
| ALT (24 h) | 86.94 ± 0.47a | 85.04 ± 1.40a | 86.58 ± 3.37a | 84.36 ± 1.31a | 99.74 ± 3.26b | 94.22 ± 0.74c | 93.16 ± 0.78c | 92.22 ± 0.44c |
| ALT (48 h) | 87.94 ± 0.57a | 87.45 ± 1.77a | 86.60 ± 1.75a | 86.84 ± 1.64a | 78.12 ± 2.2b | 85.46 ± 1.89c | 84.12 ± 1.8c | 84.80 ± 1.2c |
| AST (1 h) | 222.2 ± 3.78a | 227.8 ± 12.8a | 226.8 ± 1.95a | 228.4 ± 1.40b | 345.4 ± 7.54b | 307.0 ± 1.81c | 304.6 ± 1.91c | 310.4 ± 1.80c |
| AST (24 h) | 221.8 ± 3.62a | 229.8 ± 1.59a | 228.9 ± 1.02a | 220.4 ± 2.90b | 298.8 ± 20.1b | 256.4 ± 2.60c | 268.4 ± 3.20c | 245.8 ± 1.24c |
| AST (48 h) | 222.2 ± 3.36a | 236.6 ± 2.24a | 214.5 ± 4.30a | 222.2 ± 3.12a | 208.2 ± 9.12b | 230.4 ± 5.60c | 234.4 ± 5.56c | 281.6 ± 4.66c |

**Lipid Profile.** There was no significant effect on the serum total cholesterol CHOL levels in all experimental groups.

A significantly higher serum triglycerides (TG) and LDL levels at 1, 24, and 48 h were observed in the MPL-treated groups. However, HDL levels were significantly lower at all time intervals in the same group when compared to the control group (Table 2). Furthermore, boldine + MPL treatment group
Table 2. Influence of boldine and/or methylprednisolone on CHOL (Mg/Dl), (LDL) (Mg/Dl), (HDL) (Mg/Dl), and triglyceride (TG) (Mg/Dl) in the different rat groups.

| Groups | Parameter | Control | BOL 5 mg | BOL 10 mg | BOL 50 mg | MPL 100 mg | BOL 5 mg + MPL 100 mg | BOL 10 mg + MPL 100 mg | BOL 50 mg + MPL 100 mg |
|---------|-----------|---------|----------|-----------|-----------|------------|------------------------|------------------------|------------------------|
| LDL (1 h) | 72.00 ± 1.08a | 70.78 ± 2.34a | 71.36 ± .76a | 70.16 ± .21a | 78.54 ± .41b | 76.72 ± .81c | 76.38 ± .84c | 75.26 ± 1.25c |
| LDL (24 h) | 72.00 ± 1.08a | 72.44 ± .74a | 73.22 ± .63a | 71.28 ± 1.81a | 82.82 ± 1.05b | 76.04 ± .57c | 75.36 ± 1.48c | 73.36 ± .97c |
| LDL (48 h) | 72.00 ± 1.08a | 71.18 ± 2.13a | 71.76 ± .56a | 71.36 ± .71a | 75.94 ± 1.33b | 73.92 ± 1.65c | 74.78 ± 1.18c | 74.06 ± 1.6c |
| HDL (1 h) | 23.40 ± .24a | 23.12 ± .07a | 23.00 ± .10a | 23.16 ± .10a | 20.00 ± .31b | 21.72 ± .50c | 22.1 ± .27c | 22.5 ± .57c |
| HDL (24 h) | 25.12 ± .62a | 24.80 ± .73a | 24.96 ± .44a | 25.50 ± 22a | 19.20 ± .37b | 23.40 ± .40c | 22.96 ± .48c | 23.20 ± .52c |
| HDL (48 h) | 23.60 ± .24a | 23.10 ± .40a | 23.10 ± .40a | 23.30 ± .43a | 21.64 ± .26b | 22.40 ± .40c | 22.22 ± .13c | 22.3 ± .19c |
| CHOL (1 h) | 114.8 ± 1.42a | 115.6 ± 1.60a | 112.4 ± 1.46a | 111.6 ± 1.46a | 112.2 ± 1.35a | 113.6 ± 1.16a | 112.4 ± 1.46c | 111.6 ± .97a |
| CHOL (24 h) | 119.2 ± 1.30a | 121.6 ± 1.30a | 118.4 ± 1.30a | 116.0 ± 1.30a | 116.8 ± 1.30a | 119.6 ± 1.30a | 118.4 ± 1.30c | 117.6 ± 1.30a |
| CHOL (48 h) | 114.8 ± 1.30a | 115.6 ± 1.30a | 112.4 ± 1.30a | 111.6 ± 1.30a | 110.2 ± 1.30a | 113.6 ± 1.30a | 112.4 ± 1.30c | 111.6 ± 1.30a |
| TG (1 h) | 86.00 ± 1.89a | 84.32 ± 2.91a | 81.90 ± 1.15a | 83.72 ± 2.41a | 129.2 ± 1.65b | 117.1 ± 45c | 120.8 ± .48c | 118.3 ± .67c |
| TG (24 h) | 89.40 ± 2.22a | 85.50 ± 1.91a | 86.94 ± .77a | 88.18 ± 2.39a | 147.4 ± 1.12a | 107.0 ± 1.54a | 106.4 ± 1.66c | 105.6 ± 2.20c |
| TG (48 h) | 88.00 ± 2.36a | 89.54 ± 1.12a | 87.18 ± 2.08a | 89.11 ± 1.13a | 97.72 ± 1.54a | 94.60 ± 1.93c | 91.60 ± 1.40c | 93.80 ± 1.93c |
(BOL 5, 10, 50 mg/kg + MPL) showed significantly reduced TG and LDL levels when compared to MPL groups, while HDL levels were increased at all-time intervals. Boldine supplementation provided a significant protection against the MPL-induced elevation of TG and LDL levels in serum.

**11-Beta-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) enzyme activities.** There was significant increase in the 11β-HSD1 enzyme activities in liver tissues homogenates in MPL-treated groups at 1, 24, and 48 h when compared to control group. However, the treatment with boldine + MPL (BOL 5, 10, 50 mg/kg + MPL) significantly decreased the activities of 11β-HSD1 at all-time intervals when compared to MPL group (Table 3).

**Oxidative Stress Markers and Antioxidant Enzymes in Liver Tissue**

**Oxidative Stress Markers.** There was significantly increased lipid peroxidation (LPO) and nitric oxide (NOx) levels in the MPL treated group, while glutathione (GSH) levels were significantly decreased in the liver tissues homogenates at 1, 24, and 48 h when compared to control group. On the other hand, the administration of boldine (BOL 5, 10, 50 mg/kg) with MPL caused a reduction in oxidative stress in liver, particularly at 24 h with boldine at a dose of 50 mg/kg (Table 4).

**Antioxidant Enzymes.** The superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and glutathione reductase (GR) activity levels in liver tissues homogenates were significantly decreased in the MPL-treated group at 1, 24 and 48 h, when compared to the control group. Treatment with boldine (BOL 5, 10, 50 mg + MPL) significantly increased the levels of SOD, GSHPx, and GR, when compared to the MPL group. On the other hand, the administration of boldine (50 mg/kg) with MPL as an antioxidant had the best effect on liver, especially at 24 h, compared to other concentrations (Table 5). Boldine supplementation provided a significant protection against the MPL-induced oxidative stress at all time intervals. Depending on biochemical parameters, oxidative stress markers and antioxidant enzymes activities were discovered in the boldine (50 mg/kg) concentration at 24 h as the most suitable time and dose to complete the current study in the other parameters.

**Histopathological Examination**

According to Figure 2, histological examination of liver tissues in several experimental groups of rats in 24 h was observed by H&E staining. Control group rats (Figure 2A) and rats administrated with boldine (BOL 50 mg/kg) (Figure 2B) had normal histological structure of the hepatic lobule, hepatocytes with round nuclei and granulated cytoplasm, sinusoids, with no significant differences between them. MPL treated rats showed histopathologic alterations in liver tissues at 24 h, including loss of normal histological structure, change in nucleus shape and size, increase in pleomorphic pyknotic nuclei with irregular nuclear envelope of hepatocytes, increase in the frequency of binucleated cells, vacuolization of hepatocytes, and hyalinization of the cytoplasm. Furthermore, fatty degeneration of hepatocytes with congestion of the central vein filled with RBCs stasis was observed (Figure 2C). However, in boldine treatment with MPL (BOL 50 mg/kg + MPL), the histological abnormalities in the liver were significantly reduced, with a slight improvement in the typical architecture of the liver and less hepatic dysplasia and fatty degeneration of surrounding hepatocytes (Figure 2D). These results demonstrate that boldine (50 mg/kg) administration protected the liver from histopathological alterations caused by MPL injection.

**Molecular Analysis**

**Real Time PCR.** After 4 weeks of the experiment, the control group and the group supplemented with boldine (50 mg/kg) had no significant effect on the level of all their expressed genes throughout the 24 h time interval. In contrast, the group treated with MPL demonstrated significant decrease in the level of SOD, GPx, and 11β-HSD1 gene expressions, when compared to control group. However, concomitant boldine administration with MPL (BOL 50 mg/kg + MPL) caused significant increase in the level of SOD and GPx gene, while 11β-HSD1 gene was decreased, when compared to MPL group. It was observed that boldine significantly protected rats

### Table 3. Influence of boldine and/or methylprednisolone on 11β-hydroxysteroid dehydrogenase enzyme type 1 (11β-HSD1) Level (UI/L) in liver sample in the different rat groups.

| Groups | Parameter | Control | BOL 5 mg | BOL 10 mg | BOL 50 mg | MPL 100 mg | BOL 5 mg + MPL 100 mg | BOL 10 mg + MPL 100 mg | BOL 50 mg + MPL 100 mg |
|--------|-----------|---------|----------|-----------|-----------|-------------|----------------------|------------------------|------------------------|
| 11β-HSD1 (1 h) |           | 3.739 ± .095 | 3.847 ± .071 | 3.891 ± .139 | 3.935 ± .163 | 6.360 ± .031 | 4.335 ± .093 | 4.719 ± .130 | 5.103 ± .193 |
| 11β-HSD1 (24 h) |           | 3.689 ± .180 | 3.928 ± .029 | 3.797 ± .063 | 3.939 ± .026 | 7.503 ± .051 | 5.196 ± .194 | 5.31 ± .192 | 5.468 ± .134 |
| 11β-HSD1 (48 h) |           | 2.928 ± .144 | 2.947 ± .003 | 3.12 ± .120 | 2.884 ± .056 | 5.705 ± .074 | 4.580 ± .000 | 4.890 ± .180 | 4.209 ± .004 |
Table 4. Influence of boldine and/or methylprednisolone on malondialdehyde (Mda) levels (Nmol/G Fresh tissue), nitric oxide (Nox) levels (Nmol/G Fresh tissue) and reduced glutathione level (Gsh) (Nmol/G fresh tissue) in different rat group.

| Groups Parameter | Control | BOL 5 mg | BOL 10 mg | BOL 50 mg | MPL 100 mg | BOL 5 mg + MPL 100 mg | BOL 10 mg + MPL 100 mg | BOL 50 mg + MPL 100 mg |
|-----------------|---------|----------|-----------|-----------|-------------|-----------------------|------------------------|------------------------|
| MDA (1 h)       | 26.16 ± .218          | 25.60 ± .35a     | 24.82 ± .44a     | 24.55 ± .165a     | 55.82 ± .223b       | 44.72 ± .243c         | 41.32 ± .365c         | 42.74 ± .370c         |
| MDA (24 h)      | 24.09 ± .212          | 23.40 ± .244a    | 22.70 ± .20a     | 23.40 ± .578a     | 53.22 ± .254b       | 83.48 ± .640c         | 38.06 ± .180c         | 37.60 ± .593c         |
| MDA (48 h)      | 25.66 ± .018          | 25.20 ± .316a    | 24.80 ± .514a    | 24.60 ± .367a     | 46.96 ± .328b       | 35.23 ± .559c         | 35.06 ± .334c         | 35.55 ± .529c         |
| NOx (1 h)       | 1.370 ± .017a         | 1.366 ± .024a    | 1.386 ± .004a    | 1.360 ± .003a     | 1.728 ± .038b       | 1.566 ± .023c         | 1.554 ± .004c         | 1.504 ± .012c         |
| NOx (24 h)      | 1.336 ± .006          | 1.374 ± .002a    | 1.322 ± .004a    | 1.306 ± .008a     | 2.214 ± .010b       | 1.794 ± .004c         | 1.764 ± .010c         | 1.744 ± .00c          |
| NOx (48 h)      | 1.328 ± .004a         | 1.370 ± .006a    | 1.342 ± .004a    | 1.326 ± .002a     | 2.020 ± .020b       | 1.554 ± .002c         | 1.522 ± .015c         | 1.494 ± .020c         |
| GSH (1 h)       | 99.68 ± .279          | 99.08 ± .450a    | 100 ± .273a      | 100.5 ± 1.123a    | 59.23 ± .654b       | 85.79 ± .608c         | 82.19 ± 1.094c        | 84.13 ± 2.255c        |
| GSH (24 h)      | 94.65 ± .279a         | 97.60 ± 1.02a    | 98 ± .632a       | 100 ± 1.378a      | 55.52 ± .423b       | 91.3 ± .342c          | 90 ± 1.092c           | 90 ± .678c            |
| GSH (48 h)      | 94.66 ± .277a         | 97.60 ± .748a    | 98 ± .632a       | 99.68 ± .279a     | 58.24 ± .656b       | 89.85 ± .563c         | 89.72 ± .471c         | 86.91 ± 2.09c         |
| Groups Parameter | Control   | BOL 5 mg | BOL 10 mg | BOL 50 mg | MPL 100 mg | BOL 5 mg + MPL 100 mg | BOL 10 mg + MPL 100 mg | BOL 50 mg + MPL 100 mg |
|------------------|-----------|----------|-----------|-----------|------------|----------------------|----------------------|-----------------------|
| GSHP (1 h)       | 493.4 ± .403\(^a\) | 479.3 ± .395\(^a\) | 489.2 ± .549\(^a\) | 502.1 ± 1.184\(^a\) | 293.9 ± .813\(^b\) | 339.9 ± .616\(^c\) | 346 ± 1.378\(^c\) | 351.4 ± .36\(^c\) |
| GSHPx (24 h)     | 496 ± .570\(^a\) | 483 ± 1.095\(^a\) | 492.2 ± .621\(^a\) | 503.5 ± .317\(^a\) | 270.6 ± .534\(^b\) | 355.6 ± .98\(^c\) | 363.4 ± .892\(^c\) | 373.9 ± 2.14\(^c\) |
| GSHPx (48 h)     | 494.4 ± 1.183\(^a\) | 482 ± .857\(^a\) | 486 ± 1.095\(^a\) | 498.8 ± .122\(^a\) | 320.8 ± .849\(^b\) | 342.8 ± 1.236\(^c\) | 348.6 ± .218\(^c\) | 350.7 ± .371\(^c\) |
| GR (1 h)         | 491.2 ± .416\(^a\) | 474.3 ± .712\(^a\) | 485.3 ± .590\(^a\) | 492.4 ± 1.064\(^a\) | 420.9 ± .941\(^b\) | 443.7 ± .784\(^c\) | 434.5 ± .671\(^c\) | 447.9 ± .70\(^c\) |
| GR (24 h)        | 491.6 ± .207\(^a\) | 479.5 ± .689\(^a\) | 489 ± .836\(^a\) | 498 ± .547\(^a\) | 392.9 ± .972\(^b\) | 469.9 ± .616\(^c\) | 460.8 ± 1.428\(^c\) | 475.9 ± 1.57\(^c\) |
| GR (48 h)        | 493.2 ± .810\(^a\) | 478.9 ± .425\(^a\) | 487.3 ± 1.043\(^a\) | 496.2 ± .651\(^a\) | 418.2 ± .603\(^b\) | 453.9 ± .300\(^c\) | 449.3 ± .667\(^c\) | 455.8 ± 1.17\(^c\) |
| SOD (1 h)        | 1.840 ± .011\(^a\) | 1.772 ± .013\(^a\) | 1.822 ± .014\(^a\) | 1.896 ± .014\(^a\) | .804 ± .011\(^b\) | .876 ± .018\(^c\) | 1.890 ± .011\(^c\) | 1.772 ± .01\(^c\) |
| SOD (24 h)       | 1.940 ± .020\(^a\) | 1.866 ± .020\(^a\) | 1.914 ± .016\(^a\) | 1.950 ± .018\(^a\) | .510 ± .004\(^b\) | 1.014 ± .006\(^c\) | 1.940 ± .020\(^c\) | 1.866 ± .02\(^c\) |
| SOD (48 h)       | 1.880 ± .018\(^a\) | 1.832 ± .014\(^a\) | 1.862 ± .023\(^a\) | 1.882 ± .018\(^a\) | .830 ± .014\(^b\) | .956 ± .022\(^c\) | 1.014 ± .016\(^c\) | 1.028 ± .004\(^c\) |
against the MPL-induced by decreasing the expression of genes of antioxidant enzymes (Figure 3). Meanwhile, the GR gene was not expressed in the hepatocyte.

Biomarkers of Cell Death

DNA Fragmentation. DNA fragmentation is known as a marker of apoptosis. The qualitative measurement of the integrity of the hepatic genomic DNA has been studied by agarose gel electrophoresis (Figure 4). The DNA extracted from control rats (lane (1)) and BOL 50 mg/kg treated (lane (2)) demonstrated high-quality DNA, while MPL treatment induced DNA fragmentation at 24 h (lanes 3). However, the groups treated with BOL 50 mg/kg + MPL (lane (4)) demonstrated less DNA damage.

Figure 2. Histological examinations by hematoxylin and eosin staining demonstrating the effect of Boldine (BOL) on Methylprednisolone (MPL) induced liver damage in rats. Light micrographs of the liver of control (A) and treated group; (B) BOL 50 mg/kg b.w./day, showing the normal hepatic plates separated by blood sinusoids (BS), Endothelial cell (EN), Kupffer cell (Kc), hepatocytes with round nuclei and granulated cytoplasm, blood sinusoids open into central vein (Cv). (C) MPL showing loss of normal histological structure, increase of pleomorphic pyknotic nuclei increase binucleated cells ( ), vacuolization and hyalinization of hepatocytes. Notice, fatty degeneration of hepatocytes ( ) with congestion of central vein (Cv), which filled with RBCs stasis. (D) BOL (50 mg/kg) + MPL, showing reduced hepatic damage and regain of typical architecture to a small extent with less hepatic dysplasia and less fatty degeneration of surrounding hepatocytes, less vacuolated hepatocyte and congestion of central vein (Cv) which filled with RBCs stasis. (H and E), Original magnification is × 40, (scale bar 50 μm).

Figure 3. (A) Effect of methylprednisolone on SOD gene expression in liver. SOD mRNA levels determined by the real-time PCR method were normalized to the quantity of GAPDH mRNA. (B) Effect of methylprednisolone on GPx gene expression in liver. GPx mRNA levels determined by the real-time PCR method were normalized to the quantity of GAPDH mRNA. (C) Effect of methylprednisolone on 11β-HSD1 gene expression in liver. 11β-HSD1 mRNA levels determined by the real-time PCR method were normalized to the quantity of GAPDH mRNA.
DNA Damage by Comet Assay. MPL-treated rats induced a marked increased tail intensity and tail moment at 24 h, when compared to the control rats. Furthermore, concomitant boldine administration with MPL (BOL 50 mg/kg + MPL) significantly decreased the tail intensity and tail moment in the rats at 24 h when compared to the MPL group. However, within the control and boldine group, no significant difference was observed (Figure 5; Table 6).

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**Immunohistochemical observations of the expression of cytochrome c.** The control and boldine groups recorded a negative cytochrome c expression in the majority of the hepatocytes (Figure 6). In rats treated with MPL, cytochrome c expression occurred in the majority of the hepatocytes and interstitial stroma. In contrast, in rats treated with boldine (50 mg/kg) + MPL, cytochrome c protein expression was reduced.

**Immunohistochemical observations of the expression of IL-6 (inflammatory response markers).** The result from the immunohistochemical analysis showed no significant IL-6 expression in the liver of the control group and the group supplemented with boldine (50 mg/kg) (Figure 7). However, when the rats were treated with MPL and with boldine (50 mg/kg) + MPL, low IL-6 immunoreactivity was observed.

**Discussion**

In this study, the effect of MPL on male rats was examined by conducting hematological analysis, liver function tests (ALT and AST), lipid profile tests (total CHOL, TG, LDL and HDL), histopathological, and immunohistochemical examinations. Analysis of the oxidative stress markers (LPO, NOx, and GSH), antioxidant defense enzyme activities (SOD, GPx and GR), and their gene expression and 11b-HSD1 in liver by real-time PCR, were evaluated. Furthermore, DNA damage was used to study the possible protective role of boldine for rats.

In the rats treated with MPL, a significant decrease in WBCs, RBCs count, HGB levels, HDL level, the activity of GSH, SOD, GPx and GR, and the expression of SOD and GPx was observed, when compared to the control group. However, a significant increase in the blood platelets count (PLT), ALT, AST, TG, LDL, and the activity level of 11b-HSD1 enzyme, LPO, and NOx was observed. A non-significant change in the level of total cholesterol (CHOL) was observed. The results of immunohistochemical examination revealed that MPL increased the expression of cytochrome c but non-significant change was observed in the expression of IL-6 in the liver of albino rats at both time intervals, compared to the control group.

The increase in serum ALT and AST activity, with or without abnormalities in other liver function tests, is an indication of liver injury. These levels are typically high in the manifestation of acute or mild hepatic injury.31 In the current study, ALT and AST serum levels were significantly increased in the MPL-treated group, indicating liver damage. These results agree with previous findings by El-Sawy et al32 of a significant increase in serum ALT and ALP levels. Tovchiga reported a significant increase in plasma level of ALT and AST activities in male rats, after s/c injection of dexamethasone.33 Subramaniam et al in their study discovered a dramatic reduction in plasma level of ALT and AST activities in male rats, after s/c injection of dexamethasone.34 Boldine protected the cells from leakage of liver enzymes. Thus, the decrease in the enzyme content in serum of the boldine + MPL-treated rat is a definite indication of hepatoprotective action of boldine. The decrease in blood parameters could be attributed to the negative impact of glucocorticoids on bone marrow.32 In this study, treatment with MPL had significant effect on some of the assessed hematological parameters. The WBCs, RBCs count, and HGB levels were significantly decreased, while PLT count was significantly increased in the MPL-treated group when compared to control group. These results agree with previous findings by El-Sawy et al.32 who reported that dexamethasone
Figure 5. Effect of Boldine on methylprednisolone induced DNA damage, comet (single cell gel electrophoresis) assay (N = 3). (A) Representative micrograph of fluorescent DNA stain of control cells, showing undamaged and supercoiled DNA remaining within the unclear cell membrane. (B) Representative micrograph of fluorescent DNA stain of treated with boldine (50 mg/kg b.w.), showing undamaged and supercoiled DNA remaining within the unclear cell membrane. (C) Representative micrograph of fluorescent DNA stain of treated with methylprednisolone (100 mg/kg b.w), showing denatured DNA fragments migrating out from cell in a long comet tails. (D) Representative micrograph of fluorescent DNA stain of treated with boldine (50 mg/kg b.w.) + methylprednisolone (100 mg/kg b.w), showing mild degree of denatured DNA fragments migrating out from cell compared with time-matched controls.

Table 6. Tail intensity (% of total genomic DNA found in Th Tail Of The comets) and tail moment (tail length X Tail Intensity/100) measured with comet assay in whole blood of rats treated with boldine and/or methylprednisolone.

| Groups parameter | Tail intensity | Tail moment |
|------------------|----------------|-------------|
| Control          | 11.23 ± .943a  | 5.966 ± .206a |
| BOL 50 mg        | 10.23 ± .305a  | 4.766 ± .226a |
| MPL 100 mg       | 21.05 ± .846b  | 10.59 ± .220b |
| BOL 50 mg + MPL 100 mg | 12.66 ± .735c | 6.966 ± .302c |

(.25 mg/kg) injection induced a significant reduction in RBCs counts, PCV%, and WBCs counts, and also induced eosinopenia, lymphocytopenia, and neutrophilia. In a study by Seo et al., reduction in the number of different types of WBCs in a murine Asthma model after treatment with dexamethasone was observed. Also, in a study by Jouda, reduction in WBCs count of healthy mice after oral dexamethasone administration was observed. Elazem and Abo-Kora suggested that dexamethasone reduced the total WBCs counts and other types of WBC, which caused its immune-suppression effect. Hirotani et al. showed a decrease in leukocytes and mucosal mast cell after prednisone treatments. Furthermore, previous findings have also demonstrated glucocorticoid-induced lymphopenia to lympholysis in blood and lymphoid tissue, as well as increased lymphocyte movement from blood to other bodily compartments, or both. Also, synthetic glucocorticoids were observed to cause apoptosis of lymphocytes in rodents. According to Jain, eosinopenia and monocytopeny are caused by decreased bone marrow release, intravascular lysis, reversible sequestration in organs rich in the mononuclear phagocyte system, and increased migration in tissues due to stress caused by elevated catecholamine levels or corticosteroid administration.

In a study by Bourchier and Weston, dexamethasone treatment caused an increase in PLT count of infants with choicest lung disease, thus the number of platelets in thrombocytopenia were recovered with dexamethasone. Xu et al. in another study also observed an elevated PLT count by oral dexamethasone uptake. This could be due to a decrease in the platelet-derived growth factor by dexamethasone. In the present study, treatment with boldine significantly reversed these effects and increased WBCs, RBCs count, and HGB levels, while decreasing PLT count. In breast cancer in vitro and in vivo models, Paydar et al. found normal hematological results in the boldine (50 and 100 mg/kg)-treated group.

The increased mobilization of free fatty acid from adipose tissue, which could contribute to the development of hepatic lipidosis, could be the cause of the elevated serum lipid levels. The TG and LDL levels were significantly increased, while HDL levels were significantly decreased in the MPL-treated group, when compared to control group. These findings are consistent with the findings of El-Sawy et al. where a significant increase was observed in serum triglycerides in male albino rats after dexamethasone injection. Furthermore, Mahendran and Shyamala reported a significant increase in serum TG and LDL after injecting male albino rats with low dose dexamethasone. An increase in the activity of major fatty acid synthetic enzymes could explain the rise in blood triglycerides.

Synthetic glucocorticoids have limited therapeutic benefit due to adverse effects, including hepatic dysfunction and organ damage. Prednisolone-induced hepatic dysfunction is multifactorial, and this is linked to the disruption in the mitochondrial respiratory chain due to a significant decrease in complex I activity, a consequence of the disturbance in the mitochondrial respiratory chain. They are also considered as the major source of reactive oxygen species (ROS) and oxidative damage. In the present study, treatment with MPL had a significant effect on the activities of numerous oxidative stress markers. In the MPL-treated group, hepatic LPO and NOx levels were significantly higher, while GSH level was significantly lower, when compared to the control group. These results agree with previous findings by Hegab et al.
where an imbalance in redox status was observed after prednisolone administration, with significant higher levels of hepatic LPO and reduced levels of antioxidant enzymes. This could be related to an increase in ROS caused by mitochondrial dysfunction cytochrome P450 isoforms induction by prednisolone. Also, prednisolone treatment increased NOx and iNOS levels, which is part of the ROS induced upregulation of NF-κB, which promotes iNOS, the major inducer for cytotoxic NO\(^{52}\). NF-κB/iNOS/NO pathway activation was shown to be implicated in prednisolone-induced hepatocellular damage.\(^{53}\) Also, Bardas et al.\(^{24}\) discovered a significant increased levels of heart LPO and TBARS, as a marker of oxidative stress. After treatment with high-doses of prednisolone, researchers discovered significant decrease in GSH-Px, SOD, and CAT activities, as well as lower GSH levels, in the heart.

Recent information shed lights on the antioxidant benefits of the boldine and emphasizes its nutritional and medicinal value. Boldine is an alkaloid obtained from the boldo tree, and it has been studied for its antioxidant and radical-scavenging effects. As an aporphine alkaloid, boldine protects biological targets by preventing lipid oxidation in biomembranes, protecting against protein modification, and preserving cellular antioxidant capacity at a higher degree.\(^{10}\) Also, Heidari et al.\(^{22}\) studied the antioxidant boldine and its probable hepatoprotective action in an animal model of liver injury. NO\(_x\) overproduction causes cellular malfunction and apoptosis in a range of cell types, including hepatocytes, due to activation of NF-κB/iNOS.\(^{54}\) The findings in present study demonstrated that the apoptosis could be a factor in the hepatotoxic effects of prednisolone therapy, as measured by an increase in DNA fragmentation, comet assay, and cytochrome c expression, during prednisolone treatment, which was reduced by boldine treatment. Furthermore, it significantly increased oxidative and nitrosative stress. These results agree with previous study by Cucuireanu et al.\(^{55}\) where a relationship was observed between reactive oxygen/nitrogen species and apoptosis, as well as a link between reduced complex I activity and ROS and apoptosis.\(^{56}\) This could account for the apoptotic impact of prednisolone observed in this study and others.\(^{57}\) In a study by Qiu et al.\(^{58}\) the highest dose of boldine prevented or reduced the apoptotic index of the brain cells, which is associated with a reduction in DNA fragmentation and improved antioxidative status. Several studies have shown that boldine favors anti-inflammatory activities through interfering with the production of free radicals.\(^{59}\) After oral administration of boldine (50 mg/kg) once a day, for 7days, Pandurangan et al.\(^{60}\) reported a reduction in the degree and severity of inflammation, as well as the production of IL-6 in ulcerative colitis in male albino rats. According to numerous animal studies and its widespread use as an over-the-counter medication supplement, boldine is a relatively safe medicine.\(^{61}\) Many interesting biological activities of boldine have been carried out, and can be further explored to elucidate its use as a future healing method. Boldine administration during synthetic glucocorticoid therapy could reduce the adverse effects on normal cells and body. The purpose of this study is to demonstrate that boldine has hepatoprotective effect against liver injury caused by Methylprednisolone.

**Conclusion**

In the current study, treatment with MPL caused alterations in hematological, serum biochemical parameters, lipid profile, oxidative stress and antioxidant defined enzyme markers, gene
expression of SOD, GPx, GR and 11b-HSD1 in liver, and induced histological and immunohistochemical examinations changes, and DNA damage. Boldine was safe and efficacious in alleviating the toxicity, as well as having antioxidant and anti-apoptotic capability against cellular damage in the liver via Methylprednisolone. We recommend that boldine be included in every day diet under medical supervision. It can also be used as a supplement to synthetic glucocorticoids, as an adjuvant therapy.

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