تقم كلية الصيدلة / جامعة بغداد

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Evaluating the Effects of Chromium Picolinate (Cr+3) Supplements in Methotrexate Induced Nephrotoxicity in Rats

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Evaluation the Effects of Chromium Picolinate (Cr^{+3}) Supplements in Methotrexate Induced Nephrotoxicity in A Rat Model

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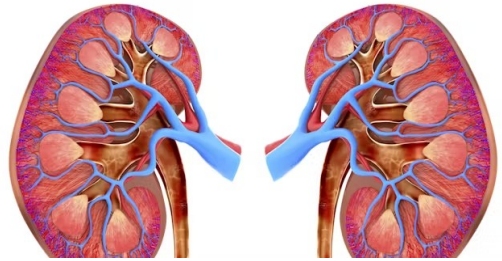
Introduction

kidneys



- **The kidneys are complex organs, and they are vital in maintaining normal body functions**
- **The most important renal functions:**

1. Regulation of water, inorganic ion balance, and acid-base balance
2. Removal of metabolic waste products from the blood and their excretion in the urine
3. Removal of foreign chemicals from the blood and their excretion in the Urine
4. Gluconeogenesis
5. Production of hormones/enzymes like erythropoietin, renin

Nephrotoxicity

- Nephrotoxicity, which is considered as the major cause for AKI, defined as a rapid deterioration in the functions of kidney because of exposure to the toxic effect of chemicals and medications.
- Different mechanisms lead to nephrotoxicity, including:

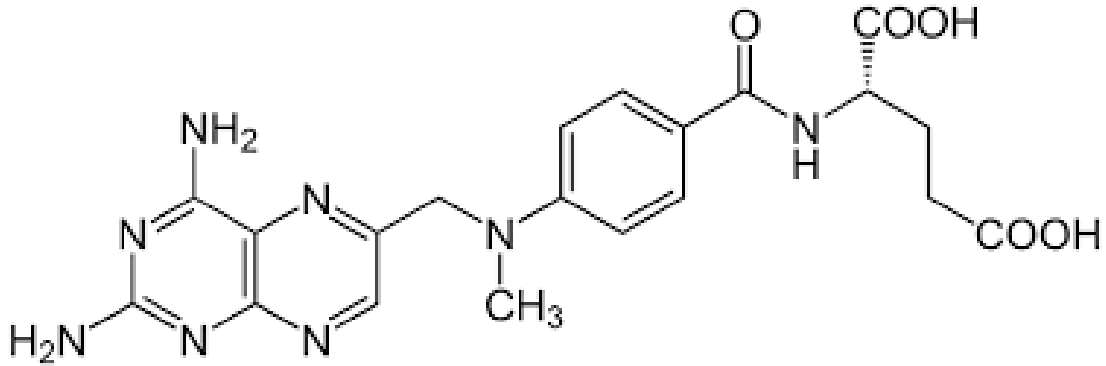
- renal tubular toxicity
- inflammation
- glomerular damage
- crystal nephropathy
- thrombotic microangiopathy

Drug-Induced Kidney Injury

Definition:

- Acute Kidney Injury (AKI) is a sudden and rapid decline in kidney function, leading to the accumulation of waste products in the blood, The diagnostic criteria for AKI include a significant reduction in glomerular filtration rate (GFR), indicated by a sudden rise in serum creatinine (sCr) levels, as well as increased blood urea nitrogen levels and decreased urine output.
- DIKI is the renal dysfunction that is induced by various medications, including MTX.
- AKI to CKD transition.
- Management of AKI include volume control, nephrotoxic drug management, fluid/electrolyte balance.

Methotrexate



- Methotrexate (MTX, 4-amino-10-methylfolic acid), is a chemotherapeutic that is classified as an "antimetabolites" drug and is a folic acid analogue and antagonist ,is employed in the treatment of autoimmune diseases due to its anti-inflammatory and immunosuppressive characteristics.

Adverse Effects of MTX Therapy

- Bone marrow suppression
- Hepatotoxicity
- Nephrotoxicity
- Mucositis

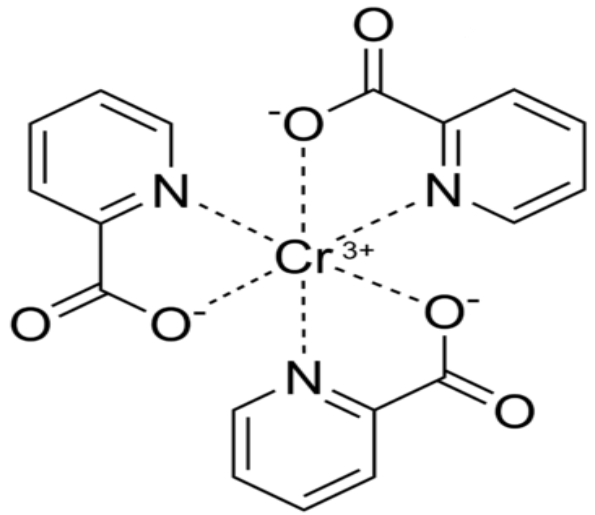
Methotrexate-Induced Nephrotoxicity

- Many cancerous patients are treated with "high-dose methotrexate (HDMTX) therapy which is linked to significant toxicities including nephrotoxicity given that the kidneys are responsible for the majority of MTX clearance
- Many pediatric and adult malignancies are treated with "high-dose methotrexate (HDMTX) therapy
- Methotrexate nephrotoxicity can lead to acute kidney injury (AKI), especially in high-dose therapy or with pre-existing kidney conditions.

Mechanisms of Methotrexate-Induced Nephrotoxicity

1. pH-dependent precipitation of MTX.
2. Direct tubular cytotoxicity (linked to mitochondrial abnormalities and oxidative stress).

chromium picolinate (CrPic3)



- is a trivalent chromium compound with a high bioavailability that is frequently prescribed to those who have issues with their metabolism of carbohydrates, such as insulin resistance and type 2 diabetes mellitus (DM2)
- Chromium has been found to have positive effects on glucose metabolism and insulin activation
- Demonstrates several therapeutic benefits, including anti-inflammatory, antioxidant.

Aims of the study

- to Evaluate chromium picolinate renal protective effects in methotrexate-induced nephrotoxicity
- Investigate the possible antioxidant effects of CrPic3 against methotrexate-induced nephrotoxicity
- Determine the possible anti-inflammatory effects of CrPic3 against methotrexate-induced nephrotoxicity

Materials and Methods

Experimental Design

- **32 adult male rats with an average body weight (130–200g)were included in the study. they were allocated equally into 4 groups each group with 8 rats:**

Group Number	Group name	Description
Group I	Control group	Rat received distilled water orally as the vehicle for eight consecutive days
Group II	Methotrexate Induction group	Rat were administered Methotrexate at the dose of 20 mg/kg intraperitoneally, as a single dose. On the first day. On the next day, the rats received distilled water for seven days
Group III	Treatment Group: Chromium $(pic)_3$ 2mg/kg	Rats were injected by Methotrexate at the dose of 20 mg/kg intraperitoneally, as a single dose on the first day. On the next day, the rats received Chromium picolinate at dose (2mg/kg body weight) orally by oral gavage for 7days.
Group IV	Treatment Group: Chromium $(pic)_3$ 4mg/kg	Rats were injected by Methotrexate at the dose of 20 mg/kg intraperitoneally, as a single dose on the first day. On the next day, the rats received Chromium picolinate at dose (4mg/kg body weight) orally by oral gavage for 7days.

After twenty-four hours from the final drug administration (day 9), blood samples were collected from the jugular vein under diethyl ether anesthesia, to obtain serum; for done biochemical test, Serum obtained for urea and creatinine levels measurement by ELISA

Then, all rats were sacrificed by cervical dislocation under diethyl ether anesthesia, and kidney tissues were isolated for analysis, each kidney was quickly-taken and rinsed by {phosphate buffer saline}solution (PBS) (pH 7.4) at 4ºC

The right kidney from each rat was fixed in 10% formaldehyde solution to be examined for histopathological changes.

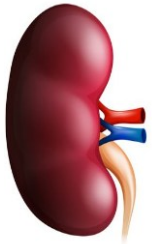
The left kidney tissue utilized for the estimation , IL-1B, MDA, KIM-1 by ELISA technique, And estimation of (SOD) and (TNF-a) by (RT-qPCR) method

sample

Day 9

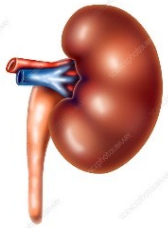
Kidney Tissue

Blood (serum)



Right kidney

Left kidney



ELISA

Histopathological examination

qPCR

TNF-alpha
SOD

ELISA

KIM-1
MDA
IL1B

BUN
Creatinine

Results and Discussion

Figure 1: Effects of chromium on serum creatinine levels.

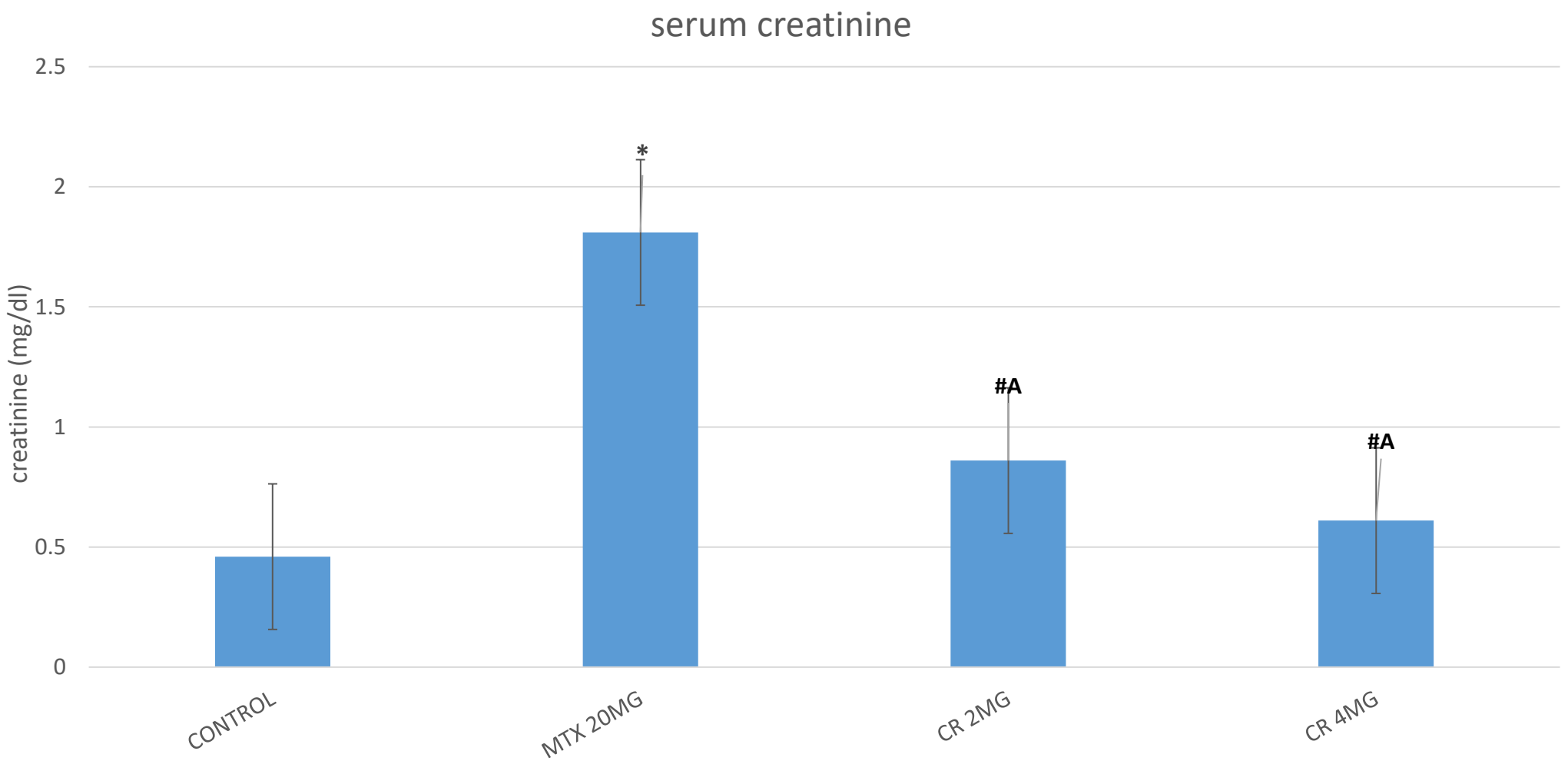


Figure 2: Effects of chromium on blood urea nitrogen (BUN) levels.

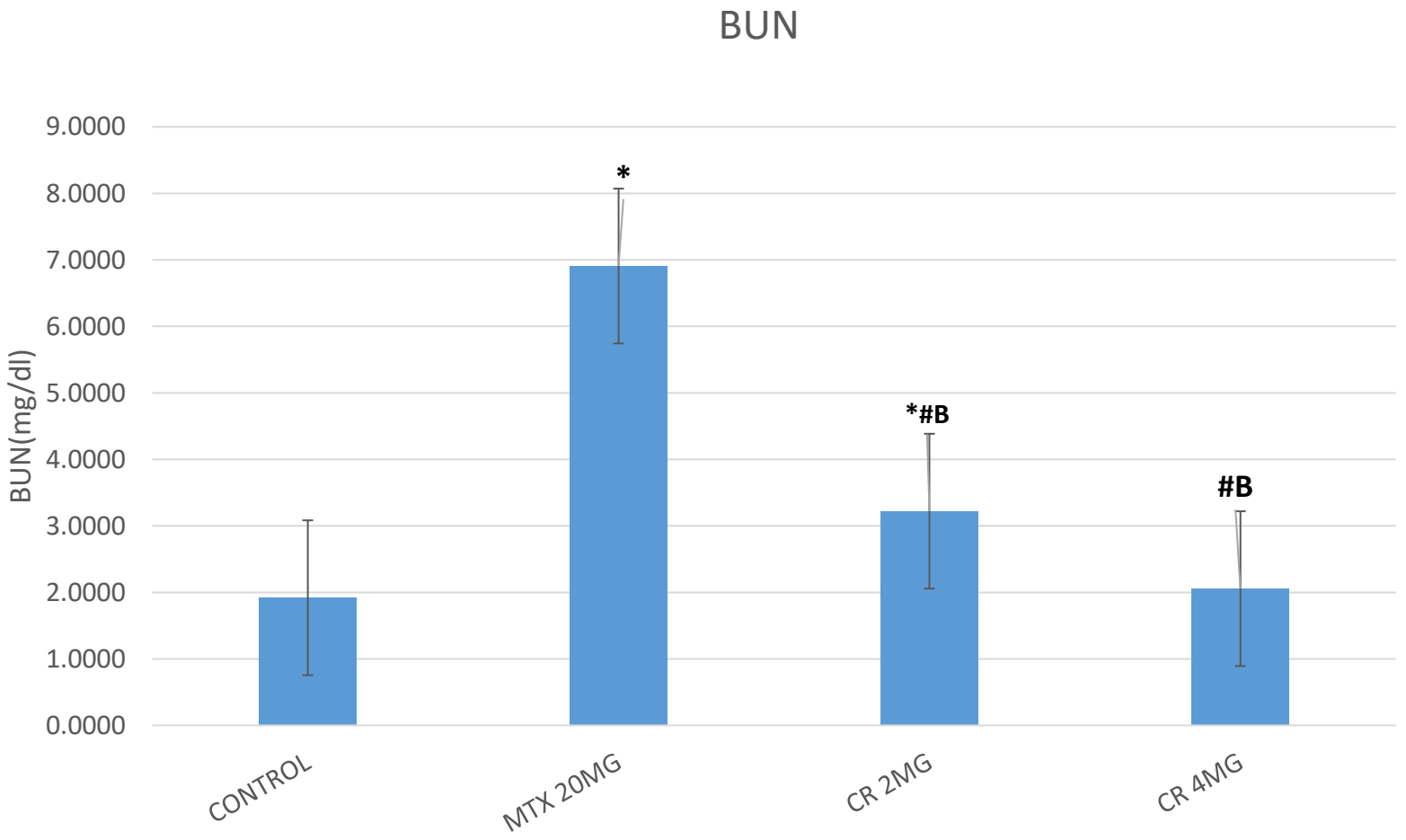


Table 3: The impact of treatment with chromium on kidney injury molecular KIM-1 levels.

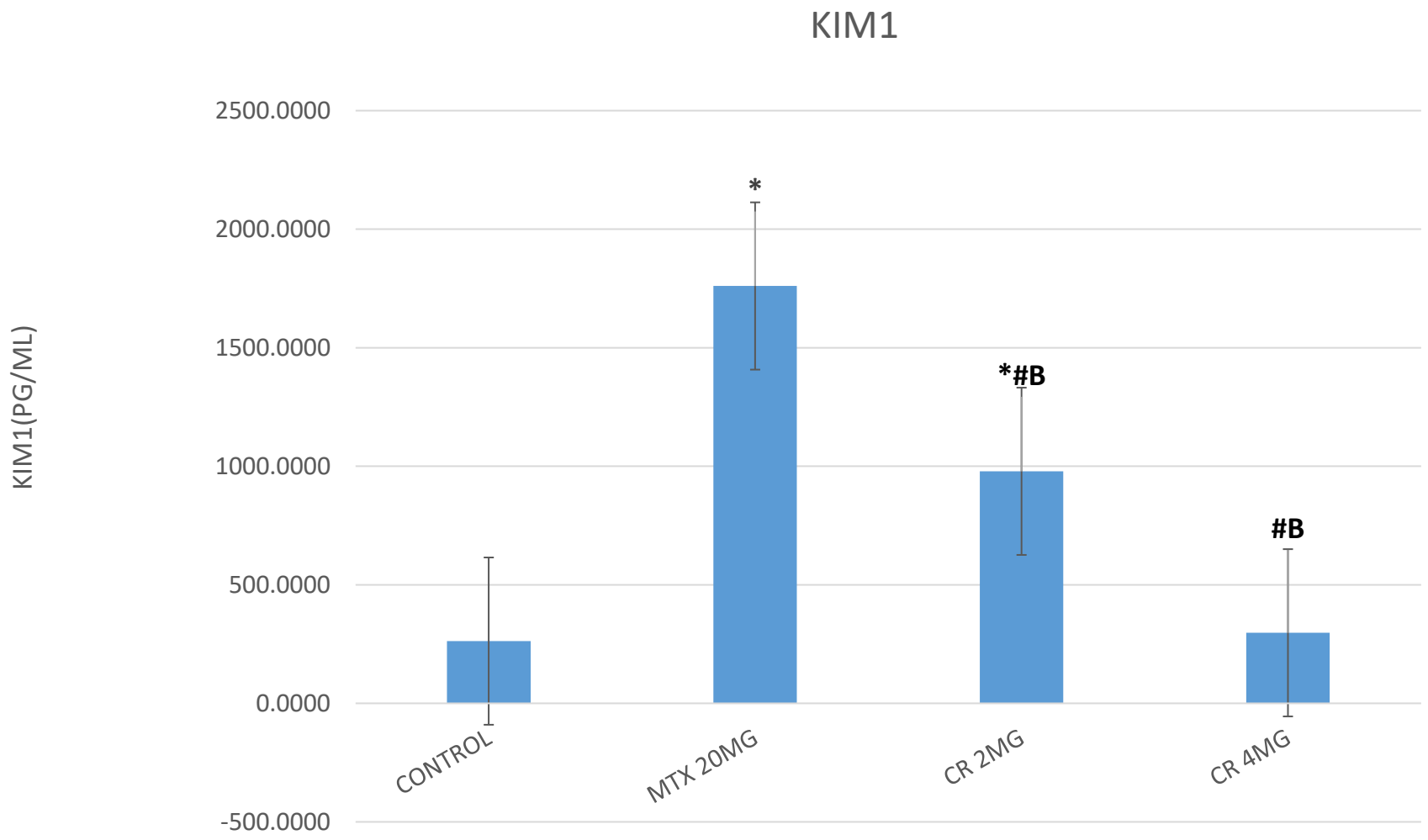


Figure 4: Effects of chromium on malondialdehyde (MDA) Level.

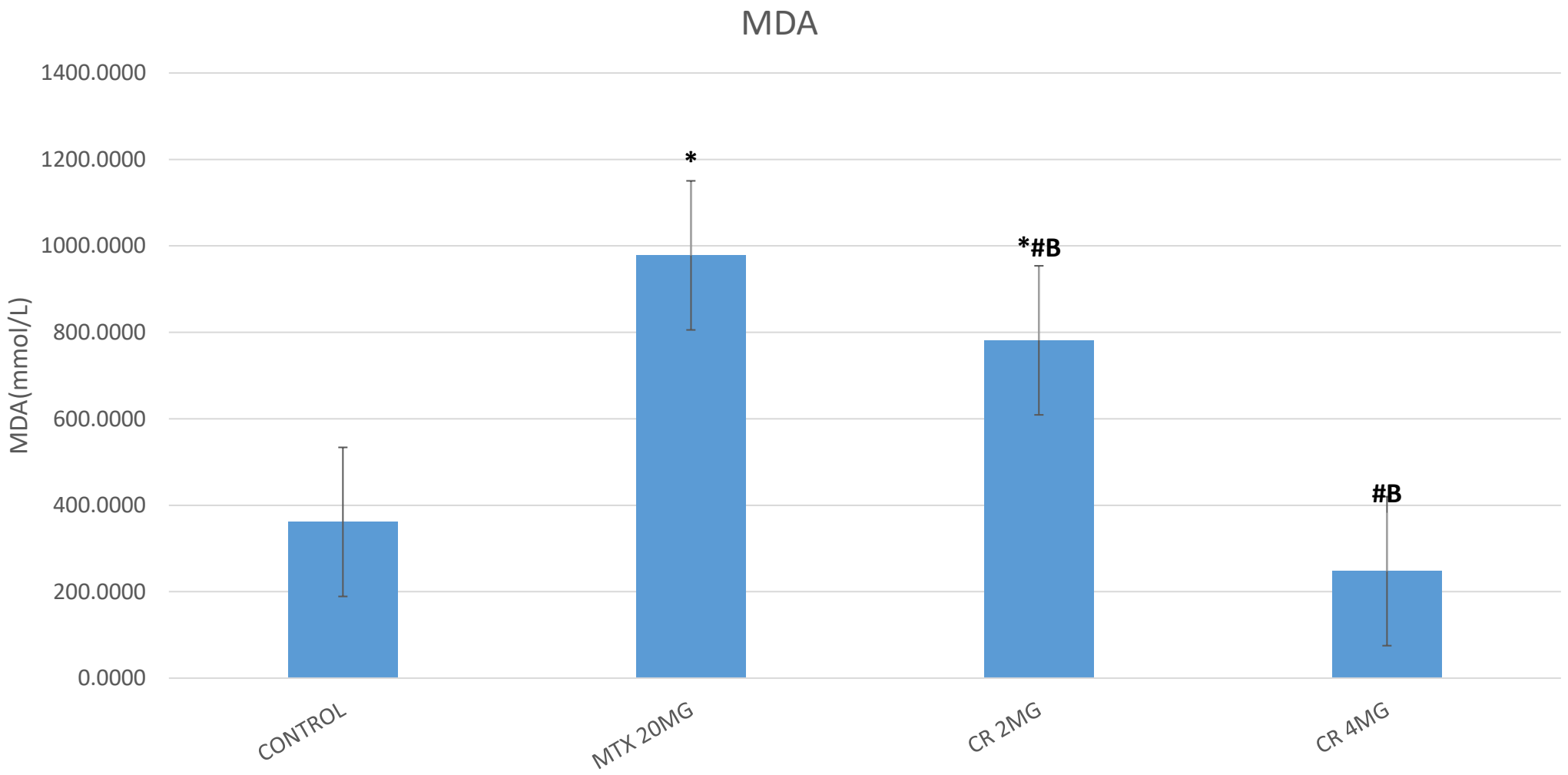


Figure 5: Effects of chromium on superoxide dismutase (SOD) expression.

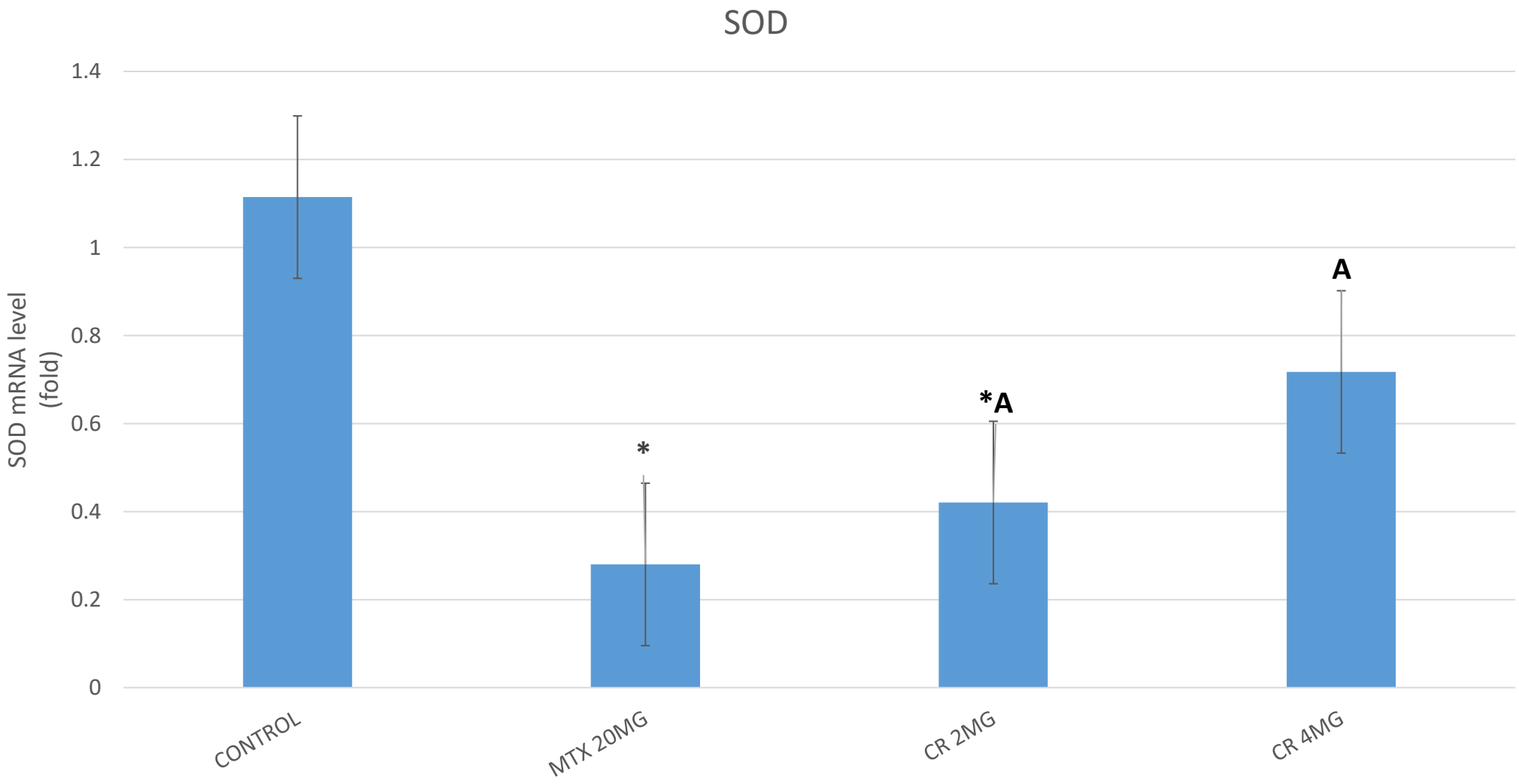


Figure 6: Effects of chromium on interleukin 1beta (IL1B) levels.

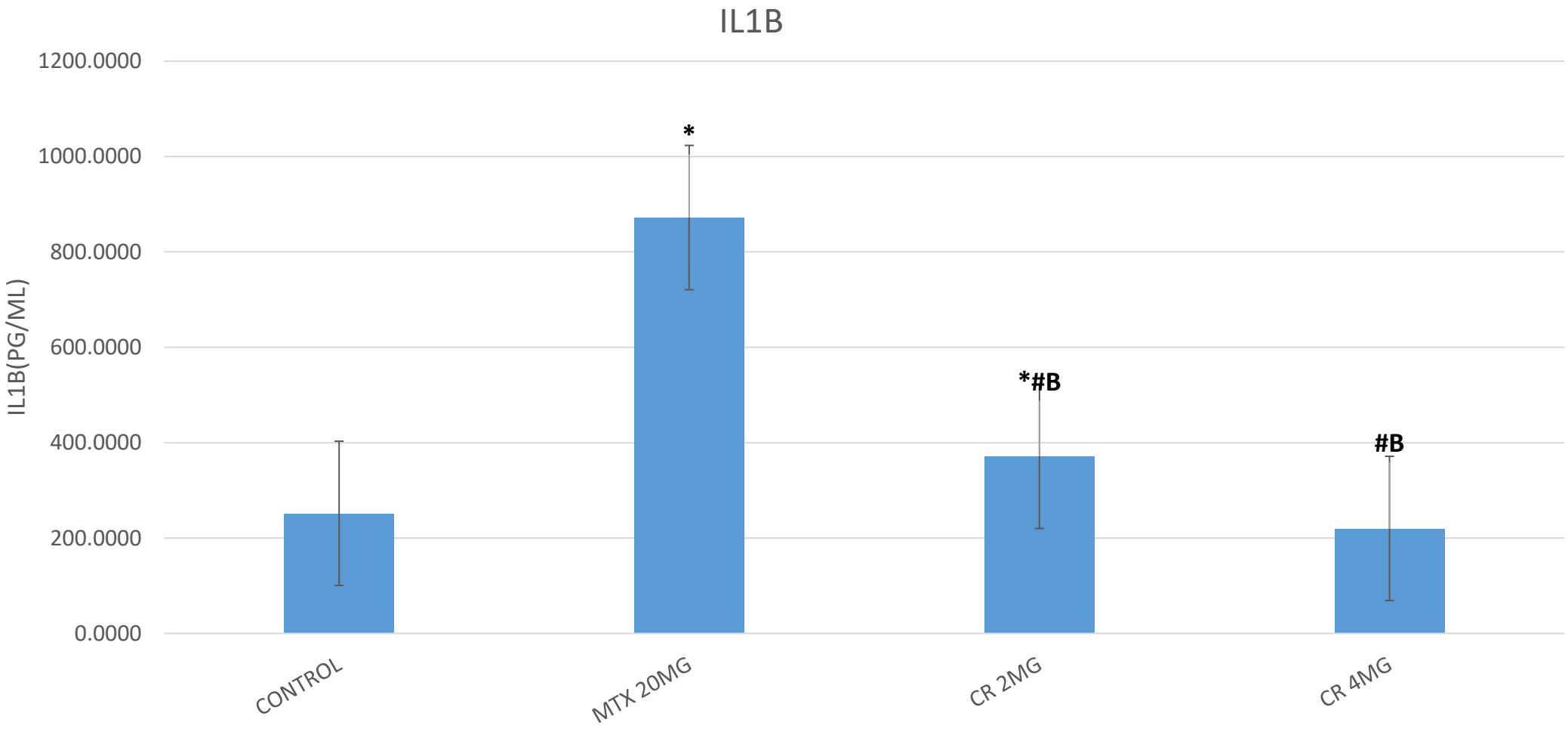
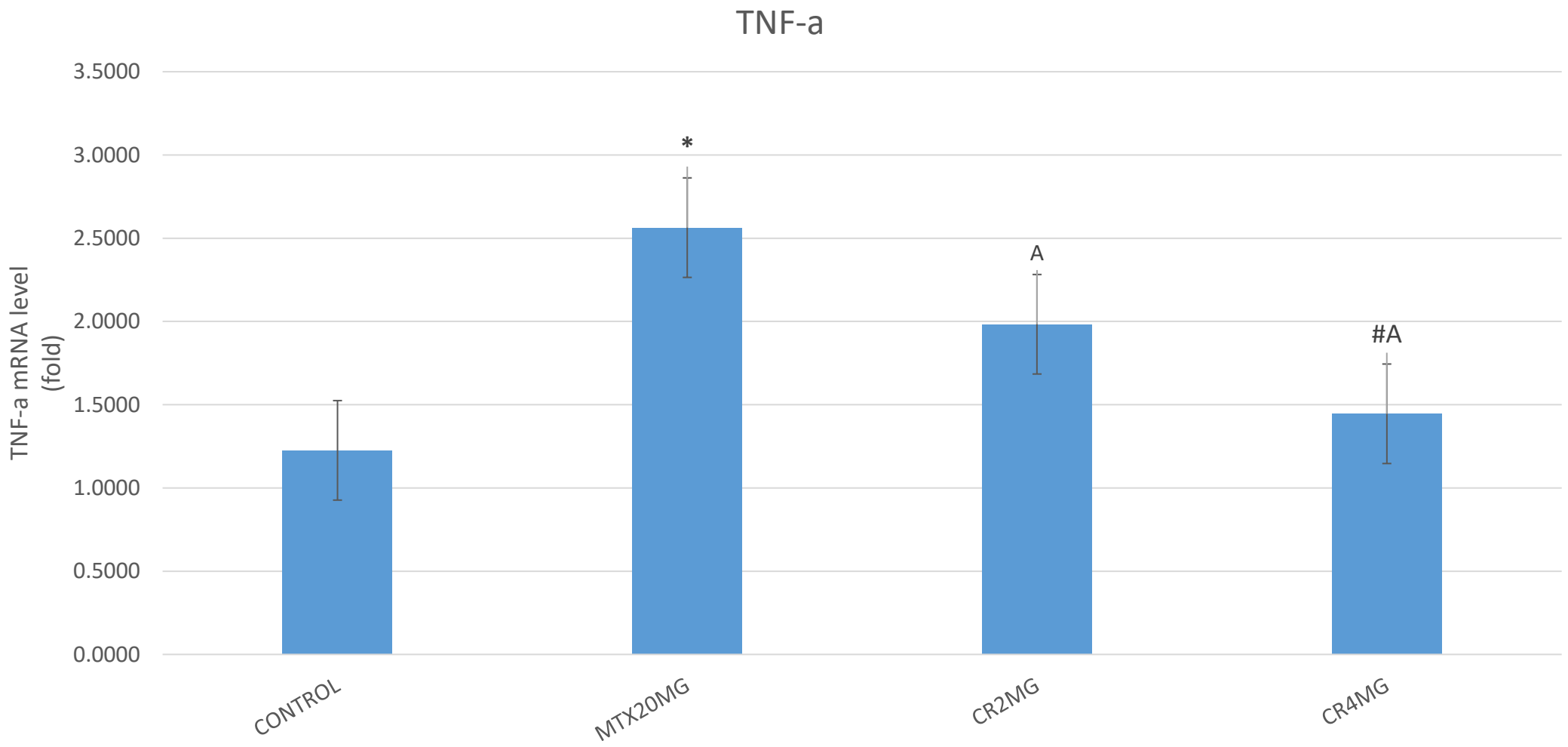
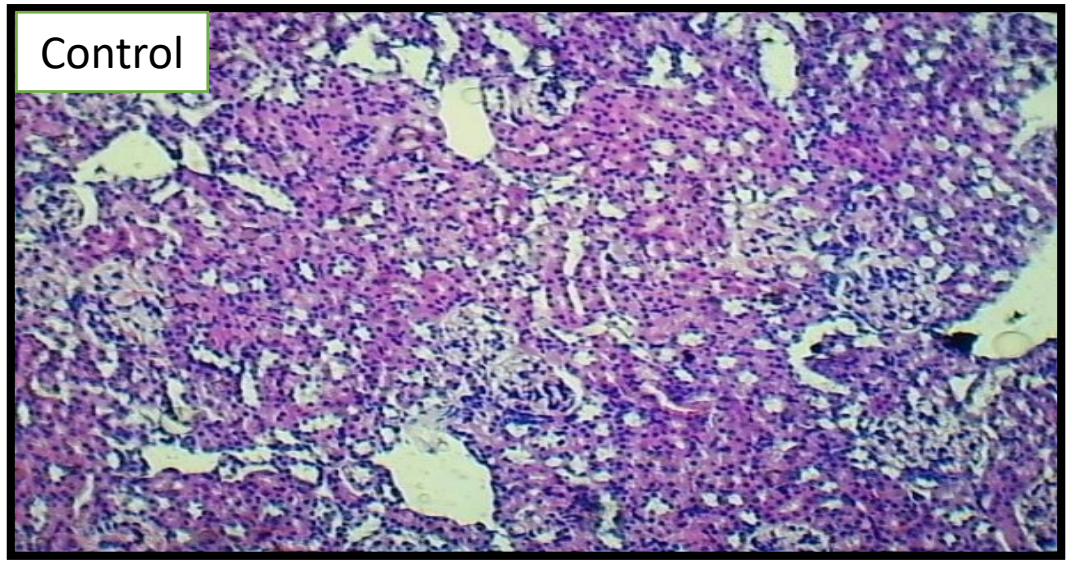
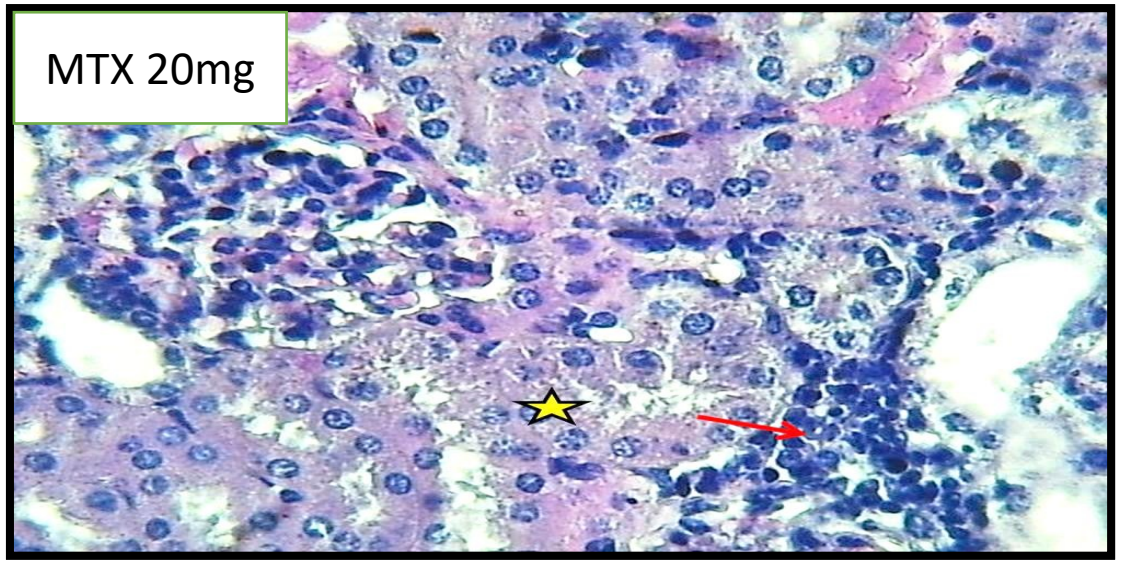


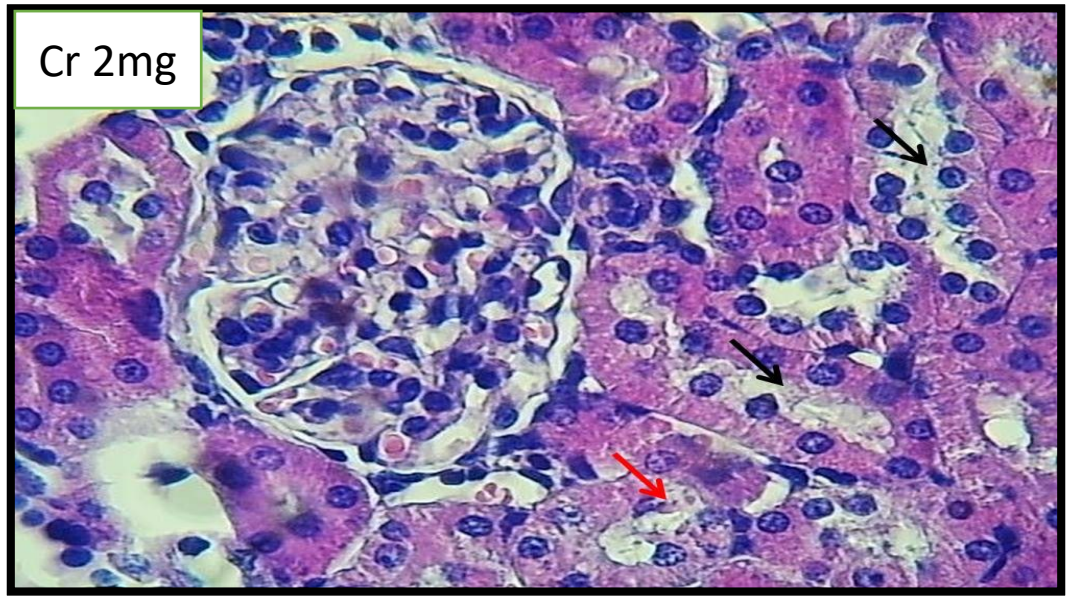
Figure 7: Effects of chromium on tumor necrosis factor (TNF-a) expression.

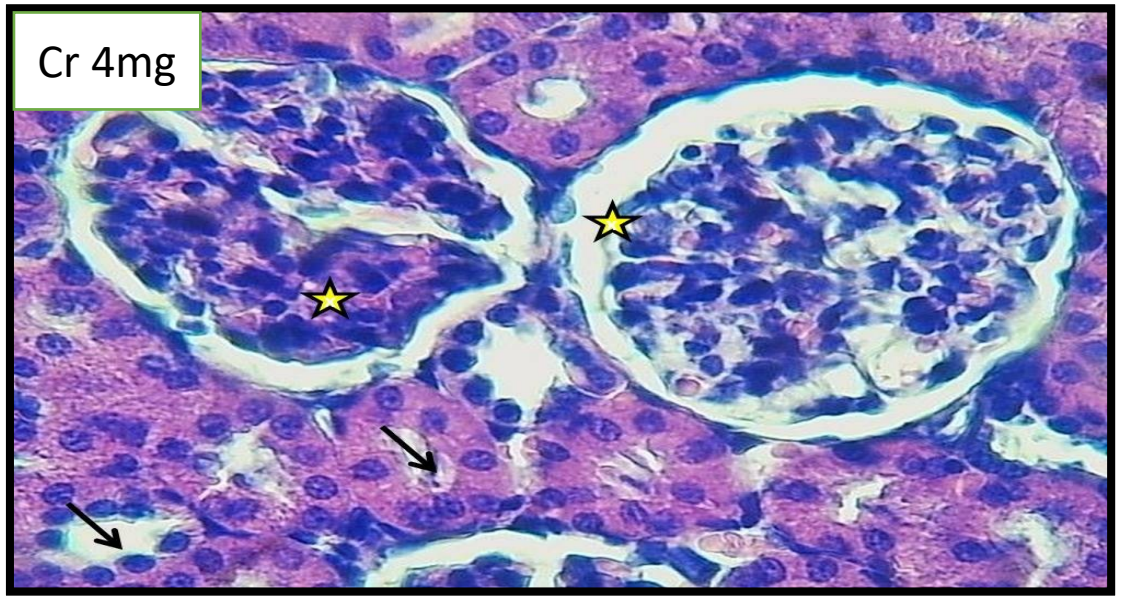


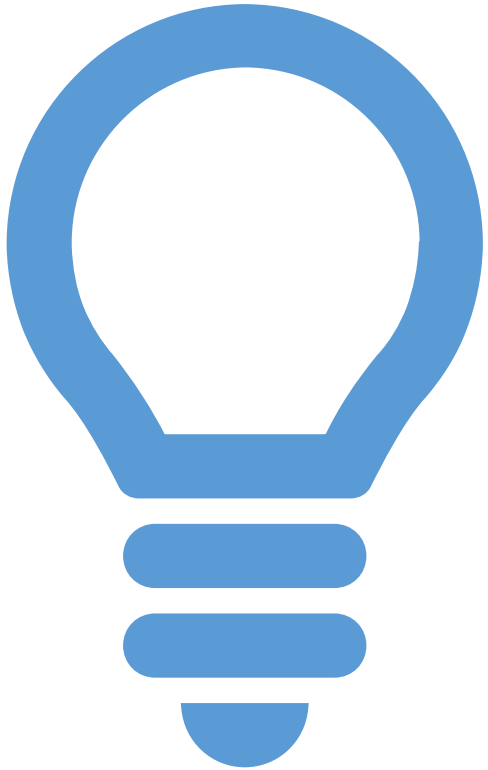
4. Effect of Cromium on kidney histology in methotrexate induced-nephrotoxicity in male rats











Conclusion and Recommendations

Conclusions

1. Chromium was effective in restoring kidney function illustrated by reduced levels of BUN and sCr.

2- Chromium mitigated the injury induced by MTX in the kidneys, evidenced by reducing the levels of the renal injury marker KIM-1.

3. Chromium showed improving effects on inflammatory cytokines by decrease TNF-α and IL-1β levels.

4. Chromium showed an antioxidant effect evidenced by improved renal tissue levels of antioxidant enzymes (SOD-1) and preventing lipid peroxidation in renal tissue, evidenced by the pronounced reduction in the renal MDA levels.

5. These results suggest that chromium has a protective effect against methotrexate-induced nephrotoxicity which is showed to be more powerful in a higher dose (4mg/kg) than the lower dose (2mg/kg) that could be attributed to its anti-inflammatory and antioxidant activity.

Recommendations for Future Work

1. Identifying the precise molecular mechanism(s) behind the antioxidant and anti-inflammatory properties of chromium.

2. Analyzing the anti-apoptotic properties of chromium against nephrotoxicity brought on by MTX.

3. Evaluating the possible therapeutic benefit of chromium in patients treated with HDMTX therapy.

4. Studying the antioxidant effects of chromium in comparison with other known antioxidants like vitamin E and omega-3.


THANK YOU!