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Evaluation of the anti-inflammatory effect of chromium picolinate in methotrexate induced nephrotoxicity rat model

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ABSTRACT: Methotrexate is a folate antimetabolite chemical used to treat many forms of cancer and cleared mainly by kidney which result in a nephrotoxicity as a major complication of methotrexate. Trivalent chromium complex, chromium picolinate (CrPic), is mostly utilized to regulate glucose and raise insulin sensitivity—particularly in diabetes. The objective of this study was to assess the renoprotective effects of Chromium in mitigating nephrotoxicity induced by Methotrexate in rats. Study conducted on 32 male rats divided into 4 groups; Group I (Control group) in which rats received distilled water orally for 8 days, Group II (Induction group) which received a single intraperitoneal injection of Methotrexate (20 mg/kg) on the first day Followed by distilled water for 7 days, Group III (Chromium 2mg) which received a single intraperitoneal injection of Methotrexate (20 mg/kg) on the first day Followed by CrPic at a dose (2mg/kg) orally for 7 days and finally group IV which is similar to Group III except that the dose of CrPic is 4mg/kg. The levels of creatinine, Kidney Injury Molecule-1 (KIM-1), interleukin 1 beta (IL-1β), and tumor necrosis factors-α (TNF-α) were evaluated for all studied groups and the results showed that the administration of CrPic caused a significant decrease ($p<0.05$) in the levels of creatinine, KIM-1, IL-1β, and TNF-α compared to methotrexate-treated rats and the levels of these markers rturned to levels comparable to those of controls. In conclusion, the administration of CrPic exhibits renoprotective and anti-inflammatory effects and may reduce the risk of methotrexate-induced nephrotoxicity.

KEYWORDS: Interleukin-1 beta; tumor necrosis factors, kidney injury molecule-1; methotrexate induced nephrotoxicity; chromium picolinate; tumor necrosis factor-alpha.

1. INTRODUCTION

Chronic kidney disease (CKD) is a widespread and advancing ailment that impacts millions of people globally. It is a chronic disorder marked by a progressive decline in kidney function over an extended period. Acute kidney damage, often known as AKI, can be identified as a rapid increase in serum (or plasma) creatinine levels and a decrease in urine output, or both clinical manifestations. Acute kidney damage is prevalent and affects over 15% of hospitalized patients, with a higher occurrence rate of over 50% in critical care units. This condition is associated with a significant death rate [1]. The primary cause of nephrotoxicity is medication use [2]. Methotrexate (MTX) is commonly used as an antimetabolite to treat many malignancies and non-neoplastic conditions, including autoimmune illnesses [3], which is accomplished by inhibiting the dihydrofolate reductase enzyme, which in turn prevents DNA synthesis [4]. Regrettably, the MTX cytotoxicity impact can also affect other malignant tissues that are not the intended target, leading to damage. Nephrotoxicity is regarded as a significant complication of MTX use since the kidney is responsible for the clearance of over 90% of the drug [5].

The use of MTX is restricted by its toxicity. Toxicity to the gastrointestinal, renal, nervous, hepatic, and bone marrow has been linked to relatively high doses of MTX [6]. Previous studies have shown that oxidative stress is a significant factor in MTX's harmful effects on the kidneys. It creates too many free radicals and stops the body's antioxidant defenses from working [7].

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Moreover, several investigations have indicated that inflammation is an initial mechanism in the nephrotoxicity of MTX [8]. Tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 are two pro-inflammatory markers that have been found to have a part in the process of kidney damage occurring. TNF-α is a crucial cytokine that promotes inflammation in the glomeruli, leading to kidney fibrosis [4]. The underlying mechanism of MTX toxicity is that, in renal cells, cell division is halted by MTX, which disrupts the normal turnover of renal epithelial cells and causes cell stress by interfering with DNA synthesis [9]. Damage-associated molecular patterns, often known as DAMPs, are chemicals that are released from cells as a response to situations that cause cellular death and stress. These molecules function as danger signals, activating innate immune cells. Activated immune and tubular cells are responsible for the secretion of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α), which are essential for amplifying the inflammatory response by attracting additional immune cells and promoting the production of additional cytokines [7, 10].

Chromium is a vital mineral necessary for metabolizing carbohydrates, lipids, and proteins [11]. Glucose tolerance factor is another name for Chromium. Through its effects on insulin sensitivity and the inflammatory system, Chromium is essential for controlling glucose and lipid metabolism. Chromium is often utilized as a supplemental treatment to enhance metabolic illnesses, including dyslipidemia and diabetes [12]. In the earlier work, it was shown that Chromium causes a reduction in TNF-α and IL-1β levels and also reduces the levels of IL-10 to levels comparable to those of normal in a mouse model of hepatic steatosis induced by oleic acid [13]. Research utilizing cell and animal models has proven that chromium supplementation affects inflammatory mediators, including IL-6 and TNF-α [14]. The precise biochemical mechanism behind the potential of chromium supplementation to enhance insulin sensitivity and reduce vascular inflammation in diabetes remains unclear. Prior research has shown that adding Chromium prevents the rise in TNF-α and oxidative stress levels in cultured monocytes when exposed to elevated glucose levels [15].

The present work aimed to assess the renoprotictive effect of chromium picolinate (CrPic) by measuring the levels of creatinine and kidney injury molecule (KIM-1) and assess the anti-inflammatory effect by measuring the levels of IL-1β and TNF-α in a methotrexate-induced nephrotoxicity male rats model.

2. RESULTS

2.1. Effects of Chromium on creatinine and kidney injury molecule-1 (KIM-1)

Results demonstrated in Table 1 and Figure 1 showed that the treatment of Methotrexate to rats at a dosage of 20 mg/kg "group II" resulted in significant elevations (P<0.05) in the levels of creatinine when compared to the group I "control group." At the same time, after chromium treatment, a significant decrease is observed when comparing group III (chromium 2mg + MTX 20 mg) and group IV (chromium 4 mg + MTX 20 mg) with the group II (MTX group) (P<0.05). Moreover, there was no significant difference between groups III (chromium 2m g+ MTX 20 mg) and IV (chromium 4mg +MTX 20 mg) (p>0.05).

Results illustrated in Table 1 and Figure 2 showed that the treatment of Methotrexate to rats at a dosage of 20 mg/kg "group II" resulted in significant elevations (P<0.05) in the levels of KIM-1 when compared to group I "control group." At the same time, after chromium treatment, a significant decrease is observed when comparing group III (chromium 2mg+MTX 20 mg) and group IV(chromium4mg+MTX 20 mg) with the methotrexate group (P<0.05). Moreover, there was a significant difference between groups III (chromium 2 mg +MTX 20 mg) and IV (chromium 4 mg+MTX 20 mg) (p<0.05).

Table 1. Levels of creatinine and KIM-1 in the sera of all studied groups

Groups	Creatinine (mg/dl) (Mean ± STD)	KIM-1 (PG/ML) (Mean ± STD)
Group I Negative control group	0.46±0.31 a	262.48±132.22 a
Group II MTX group	1.81±0.43 b	1760.41±101.38 b
Group III Chromium 2mg +MTX	0.86±0.13 a	978.75±72.58 c
Group IV Chromium 4mg +MTX	0.61±0.19 a	297.58± 60.47 a

Levels having different letters within similar columns are significantly differences (p<0.05).

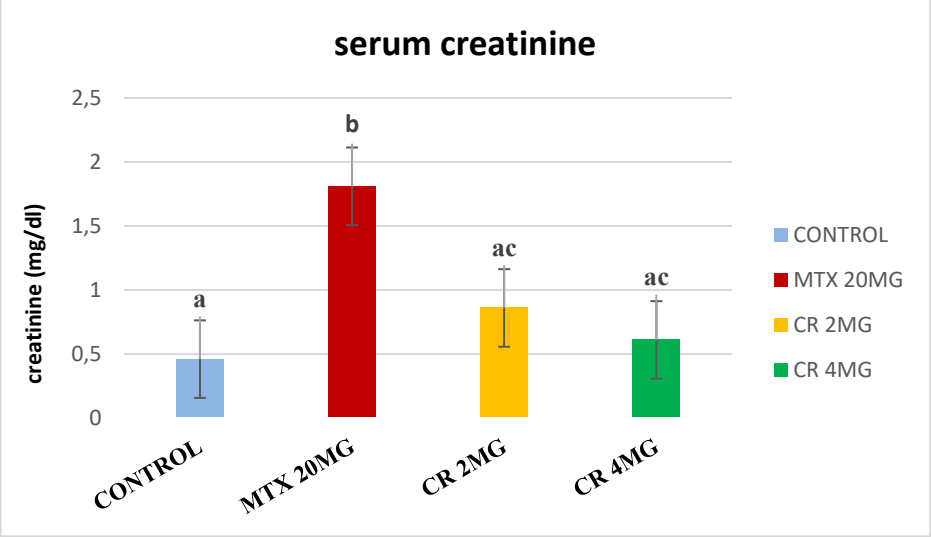

Figure 1. Chromium's effect on serum creatinine levels in all studied groups.

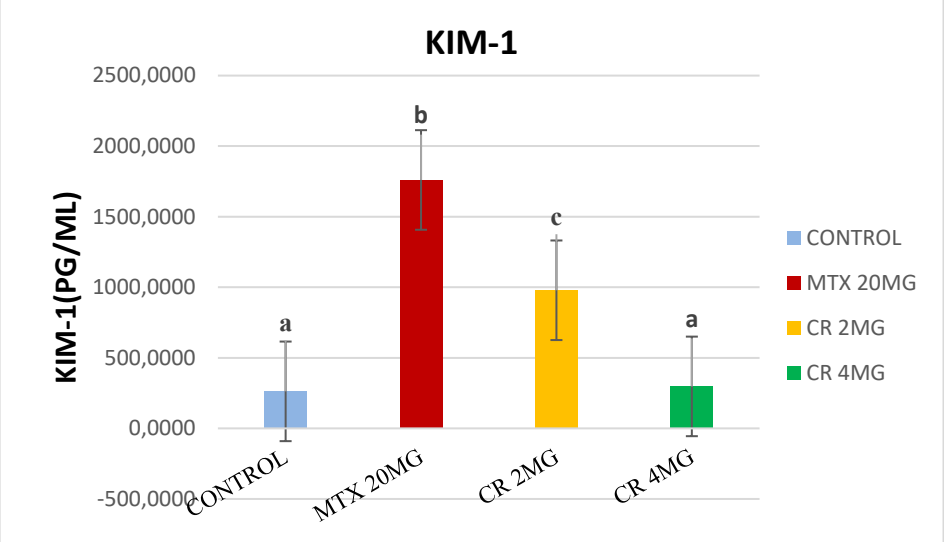

Figure 2. Effects of Chromium on kidney injury molecular (KIM-1) levels in all studied groups.

2.2. Effects of Chromium on Renal Inflammatory Parameter (IL-1β and TNFα)

Results illustrated in Table 2 and Figure 3 showed that rats administered 20mg/kg Methotrexate (group II) demonstrated a significant increase (P<0.05)in the levels of IL-1β in comparison with controls. At the same time, after chromium treatment, a significant decrease is observed when comparing group III (chromium 2mg + MTX 20mg) and group IV(chromium 4 mg+ MTX 20mg) with the MTX group (P<0.05). Moreover, there was a significant difference (p<0.05) between rats who received chromium 2 mg+ MTX 20mg (group III) and those who received 4 mg of chromium and 20 mg of MTX (group IV).

With a manner nearly comparable to that of IL-1β, the levels of TNF-α expressed were significantly increased (P>0.05) in Group II, in which rats received 20mg/kg Methotrexate compared to those in Grop I (controls) as illustrated in Table 2 and Figure 4. At the same time, no significant difference (P>0.05) was observed when comparing group III (chromium 2mg + MTX 20mg) with MTX group II. Interestingly, in the same Table, there was a significant difference in the expression of TNF-α in group IV (chromium4mg + MTX 20mg) in comparison to group II (P<0.05). Moreover, results also showed non-significant differences (P>0.05) between group III (Chromium 2mg+ MTX 20 mg) and IV (chromium 4 mg +MTX 20 mg).

Table 2. Levels of IL-1β and mRNA expression of TNF-α in all studied groups

Groups	IL-1β (PG/ML) (Mean ± STD)	TNF-α mRNA (folds) (Mean ± STD)
Group I Negative control group	251.79±108.75 a	1.22±0.86 a
Group II MTX group	871.98±56.84 b	2.56±0.77 b
Group III Chromium 2mg +MTX	371.40±26.84 c	1.98±0.72 abc
Group IV Chromium 4mg +MTX	220.12±5.61 a	1.29±0.18 ac

Levels having different letters within similar columns are significantly differences ($p<0.05$).

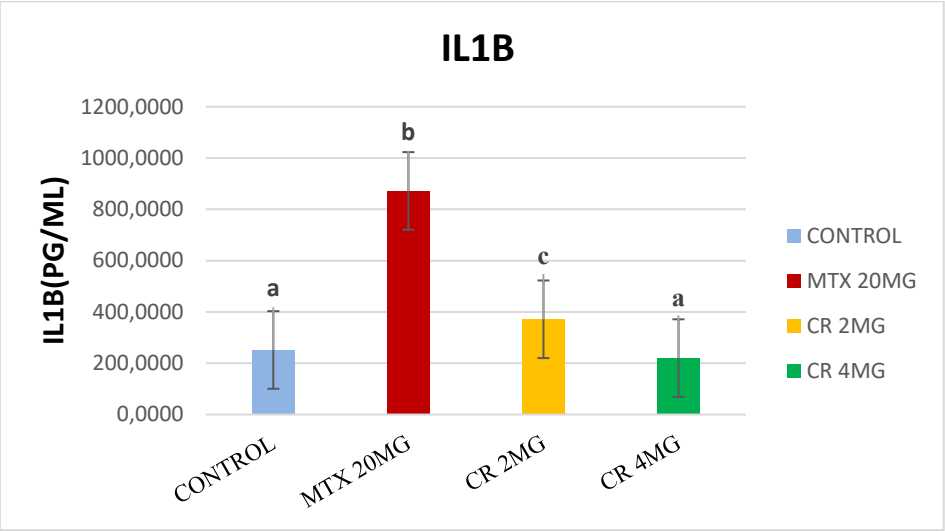

Figure 3. Effects of Chromium on interleukin-1beta (IL-1β) levels in all studied groups.

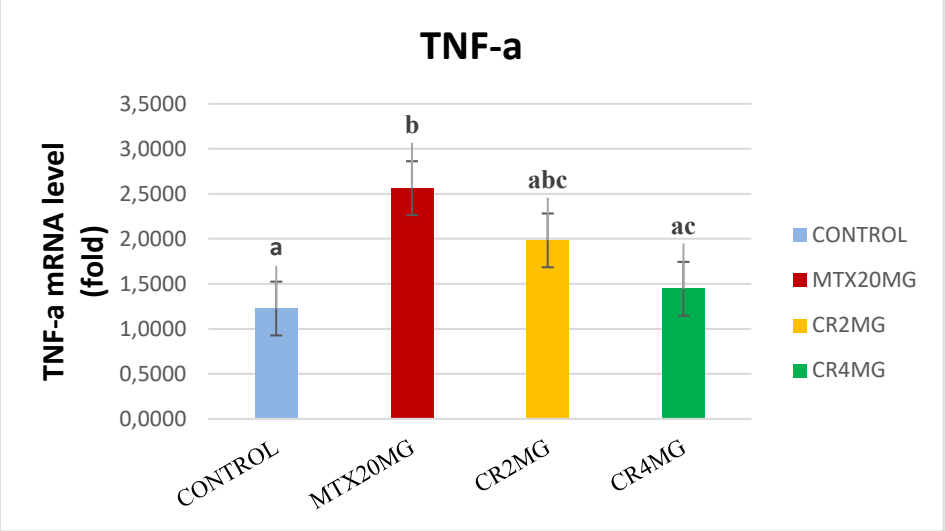

Figure 4. Effects of Chromium on tumor necrosis factor (TNFα) expression in all studied groups.

3. DISCUSSION

Presemt investigation discovered that administering Chromium after MTX therapy successfully alleviated kidney injury by decreasing inflammation caused by Methotrexate. Acute kidney injury (AKI) is a prevalent renal consequence in individuals with cancer, leading to a worse prognosis, stoppage or discontinuation of ongoing anti-cancer treatment, extended hospital stays, and heightened healthcare expenses [16].

Methotrexate (MTX) is a very effective chemical compound that acts as an antimetabolite of folate. It is utilized in the medical field to treat several types of malignancies, autoimmune illnesses, inflammatory disorders, psoriasis, rheumatoid arthritis, and other similar conditions. Although MTX has therapeutic efficacy, it is restricted in its usage since it is toxic to several organs [17]. A critical concern associated with the

administration of high-dosage Methotrexate is the occurrence of acute renal damage. The elevation of blood creatinine levels in children with Acute Lymphoblastic Leukemia is linked to a reduction in the elimination of Methotrexate following a high dosage infusion [18]. KIM-1 is a glycoprotein that spans across the cell membrane. Recent studies have demonstrated that a serum elevation of The KIM-1 level can function as an indicator of renal injury in both mice and humans [19]. Additionally, IL-1β and TNF-α are the primary pro-inflammatory cytokines. Their excessive secretion leads to the production of reactive oxygen species (ROS) and an increase in other pro-inflammatory cytokines [20].

Methotrexate results in the generation of ROS and the release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. These are the key reasons that contribute to the nephrotoxicity of Methotrexate [21]. In this current study, the administration of MTX to rats in group II resulted in a substantial increase in blood creatinine levels and KIM-1 compared to group I (control group). The findings were consistent with previous studies and demonstrated the presence of kidney damage following methotrexate therapy [22, 23]. This study shows that using CrPic for seven days after MTX treatment led to a considerable reduction in the levels of serum creatinine and KIM-1 at the same time in the CrPic group compared to the MTX group. This study provides evidence that CrPic can reduce the negative effects of MTX on renal function in rats. The results of the present work go in parallel with previous study which demonstrated that CrPic has a renal-protective effect as it cause a reduction in the levels of creatinine in rats with diabetic nephropathy. It was demonstrated that Eight weeks of CrPic supplementation was found to repair renal function and reverse renal pathological changes (renal interstitial fibrosis and glomerular sclerosis) in diabetic nephropathy rats by an antioxidative stress mechanism, as well as by inhibiting TGF-β1 and SMAD 2/3 expressions [24]. Additionally, another study conducted on diabetic male mice demonstrated that high doses of CrPic causes moderate reduction in albuminuria with a slight improvement in the creatinine clearance [25].

Moreover, MTX administration to rats in group II led to a significant elevation of TNF expression and IL-1β level compared to group I (control group). The results were in line with prior research and showed the existence of renal injury after methotrexate treatment [21]. Upon comparing the CrPic group to the MTX group, the findings of this study indicate that the CrPic group exhibited a noteworthy reduction in TNF and IL-1β levels following seven days of administration of CrPic following MTX therapy. The present investigation implies that CrPic has anti-inflammatory effects as a defense against nephrotoxicity which is also supported by previously published reviews which reported that Chromium can play anti-inflammatory roles by inhibiting nuclear factor kappa B (NF-kB) activation and attenuating insulin resistance which results in decreasing pro-inflammatory cytokines including TNF-α, IL-6, and C-reactive protein (CRP) levels in diabetic rats [26,27]. Another reviewe article reported that chromium reduced the content of pro-inflammatory cytokines (IL-1β and TNF-α, IL-12) and restored the level of anti-inflammatory cytokine (IL-10) to the control values in hepatic steatosis mice [28].

4. CONCLUSION

It was concluded that Chromium may have a renoprotictive effect against methotrexate-induced kidney damage that appear clearly in its effect on the levels of creatinine and KIM-1 with an anti-inflammatory effect that obviated by its effect upon the levels of IL-1β and TNF-α. these findings showed a promising role of Chromium renoprotictive effect agent.

5. MATERIALS AND METHODS

5.1. Drugs

Methotrexate (50mg/5ml) was supplied from Pfizer, USA, and Chromium was supplied from Source Natural, USA.

5.2. Animal

For this study, 32 adult male rats weighing 150-200g were kept in cages at the Animal Experimental and Scientific House in the College of Pharmacy, Baghdad University. The rats were maintained under controlled humidity, temperature, and light periodicity conditions, with a 12-hour cycle of light and dark. The participants were given commercially available food and drinkable water as required throughout the trial.

5.3. Experimental protocol

The study got permission from the Scientific and Ethical Committees of the Baghdad University - College of Pharmacy. Thirty-two adult waster rats were separated into four groups, including eight animals. Each group was allocated distinct treatments in the following manner:

- **Group 1 (Control group**): The rat was administered distilled water orally as a vehicle for eight consecutive days.
- **Group 2 (Induction group):** The rats were given a single intraperitoneal injection of MTX at a dose of 20 mg/kg on the initial day [29]. On the following day, the rats received distilled water for seven days.
- **Group 3 (CrPic)** $_3$ **2mg+ MTX 20 mg):** The rats were given a single intraperitoneal injection of MTX at a dose of 20 mg/kg on the initial day. The following day, the rats received CrPic at a dose (2mg/kg) by oral gavage for 7 days [30].
- **Group 4 (Chromium (pic)** $_3$ **4mg+ MTX 20 mg):** The rats were given a single intraperitoneal injection of MTX at a dose of 20 mg/kg on the initial day. The following day, the rats received CrPic at a dose (4mg/kg) by oral gavage for 7 days [30].

5.4. Sample collecting and renal tissue homogenate preparation

Twenty-four hours after the final dosage of the medicine, following the sacrifice, blood samples were taken from the jugular vein (near the throat or neck) under the effects of diethyl ether anesthesia [31]. The blood sample was transferred into a Serum separator tube and kept at room temperature ~ 25℃ for 30 minutes to clot. Then, it was centrifuged at 3000 rpm /15 min to obtain serum. The supernatant was then transferred as 250 µl aliquots into appropriately labeled microcentrifuge tubes and kept at −20°C [32]. The preserved serum samples were used to quantify the concentrations of creatinine.

Next, All the rats were killed by dislocating their necks while they were under the effects of diethyl ether anesthesia. They were following the euthanasia of the animals. The kidney was quickly removed, thoroughly washed with extremely cold phosphate buffer saline (PBS) to remove any extra blood, and then weighed and cut into little pieces. The kidney tissue was then homogenized by adding 0.9 mL of PBS (pH 7.4) to a tube containing 0.1 g of kidney tissue. Following homogenization using a homogenizer, the materials were centrifuged for 10 minutes at 10000 rpm in a chilled centrifuge 4^{o}C. The fluid supernatant was then collected and stored at -20^{o}C till analysis. The supernatant was used to evaluate kidney injury molecular (KIM-1) and interleukin 1beta (IL-1β) levels using the ELISA technique and kits purchased from (MyBioSource) according to manufacturers' procedures. And estimation of TNF-α by (RT-qPCR) method [33].

5.5. Gene expression analysis

Gene expression study of TNF-α was conducted by quantifying the levels of mRNA in kidney tissue using a common method termed "quantitative reverse transcription-polymerase chain reaction" (qRT-PCR) to quantify the mRNA levels in kidney tissue [34]. The TransZol Up Plus RNA Kit (TransGen, biotech) was used to separate total RNA from kidney lysates that had been mixed with TRIzol. Following that, the EasyScript® one-step gDNA removal and cDNA synthesis (TransGen, biotech) technique was used to synthesize complementary DNA (cDNA) [35].GAPDH was used as a control gene in SYBR Green Supermix (TransGen, biotech) to measure the amounts of mRNA expression. The sequence of the primers used for GAPDH and TNF-α shown in "Table 3".

Table 3. The sequence of the primers used in this study

Primer	Sequence 5′→3′ direction
GAPDH Forward	CGGGTTCCTATAAATACGGACTG
GAPDH Reverse	CCAATACGGCCAAATCCGTTC
TNF-α Forward	AAATGGGCTCCCTCTCATCAGTTC
TNF-α Reverse	TCTGCTTGGTGGTTTGCTACGAC

5.6. Biochemical analysis

Creatinine levels were quantified in sera using the ELISA technique. The kits were utilized in strict accordance with the instructions provided by their producers. The levels of IL-1β and KIM-1 were quantified in the kidney tissue homogenate using the sandwich ELISA technique, following the instructions provided by the kit's manufacturer [36].

5.7. Statistical analysis

Statistical analysis was performed using "the Statistical Package for Social Sciences software (SPSS)" version 25, and the results were expressed in the form of Mean ± standard deviation (STD). A one-way ANOVA with Tukey's post hoc test was used to compare data from different groups. Statistical significance was determined at a level of $P<0.05$ across all groups [37,38].

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