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Original Research Article

Probing the Nephroprotective Potential of Chrysin against Methotrexate-Induced Tubulointerstitial Nephritis and Oxidative Damage

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ABSTRACT

In a bid to treat cancer, there may be a possibility of adverse consequences for tissues such as the kidneys. Methotrexate is an effective drug of choice used in high dosages as a first-line treatment in the management of cancer. Nevertheless, it can induce tubulointerstitial nephritis (TIN), which may finally result in renal failure. Therefore, there is need to identify substances that can alleviate and protect the kidneys from such TIN, and, by extension, provide a safe environment to be co-administered with methotrexate. The study aimed to probe the nephroprotective efficacy of chrysin on the kidneys against methotrexate-induced TIN. A total of 25 adult female Wistar rats with an average weight of 200 g were randomly segregated into five (A-E, n = 5). Group A was allowed feed and water only, serving as the normal control. Group B received 20 mg/kg bw of methotrexate only. Group C received chrysin (100 mg/kg bw). In groups D and E, methotrexate administration was followed by chrysin treatment in low (50 mg/kg bw) and high (100 mg/kg bw) dosages, respectively. The results obtained from the study demonstrated significant attenuation of serum urea, creatinine, and electrolyte disturbances, attenuation of oxidative stress (superoxide dismutase, glutathione peroxidase, and catalase), and lipid peroxidation via malondialdehyde optimization while preserving the histoarchitecture of the kidneys of rats treated with chrysin in high dosage. The extrapolation from this study proposes the nephroprotective effect of high-dose chrysin against methotrexate-induced tubulointerstitial nephritis by suppressing oxidative stress and inflammation in the renal parenchyma.

Keywords: Chrysin, Methotrexate, Electrolyte disturbances, Oxidative stress, Tubulointerstitial nephritis

Introduction

As wonderful as chemotherapy may be, care must be taken in its application due to its various side effects.1 Drug-induced nephropathy is fast becoming one of the major drawbacks of the use of high-dose methotrexate in chemotherapy. Methotrexate (MTX) is a folic acid structural analogue used against a wide range of diseases, including malignancies, autoimmune diseases, and even in the treatment of ectopic pregnancies.[1,2] It is excreted primarily by the renal system.[3] The renal system has a whopping 25% involvement in the human circulatory system, to mention a few of its roles. It is noteworthy to understand that different forms of renal function impairment are frequently observed in cancer patients before, during, and after chemotherapeutic interventions.[4] It could be a result of complications from the malignancy or co-morbidities existing before chemotherapy, or the side effects and toxicity of the chemotherapeutic agents. These functional impairments could be reversible injuries or permanent damage to the urinary system – nephrotoxicity.[5]

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In recent years, a branch of medicine that deals with the study of cancer to enhance early diagnosis, treatment, and prevention – oncology has made proactive progress due to the increasingly frequent occurrence of cancer in identifying multidisciplinary measures as the most effective approach to managing cancer.[6] In the quest to improve the quality of in the human body. The most commonly affected organ in the urinary observed in cancer patients is tubulointerstitial nephritis (TIN), a group of immune-mediated inflammation that affects the tubules of the kidneys and the tissues that surround them, which can lead to fibrosis. It is a frequent cause of acute kidney injury and can result in chronic kidney disease. [9,10] TIN could be drug-induced in the setting of high-dose methotrexate as a chemotherapeutic agent. Methotrexate is a drug commonly seen and used in clinical settings, which makes it precarious that a drug so versatile and effective can also give life for cancer patients, oncology includes specialized disease management such as nephro-oncology care.[7] This is an indispensable discipline due to the role of the renal system rise to a deleterious effect: tubulointerstitial system is the kidney, and according to the rule of thumb in oncology, the extent of renal damage is crucial for the feasibility of initiating or furthering chemotherapy.8 An example of nephrotoxicity nephritis.[11]

The underlying mechanism of nephrotoxicity caused by methotrexate treatment remains unknown. However, in a study reported by Shirali & Perazella, it is understood that NADP malic enzymes are inhibited by methotrexate crystallization and precipitation in the renal tubules.[12] This inhibition decreases the availability of NADPH in cells that are used by glutathione reductase to maintain reduced glutathione. Reduced glutathione content in the cells is a known important protective agent against reactive oxygen species (ROS), thus, this renders the cells more sensitive to oxidative stress.[2] This discovery has stimulated many researchers to examine ways to mitigate the oxidative stress caused by methotrexate on the renal tissues. There is thus a

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focus on alternative therapy, of which natural products such as chrysin have become a major focus.

Chrysin (5,7-dihydroxyflavone) is an organic compound known as flavone. It is a natural component found in numerous crops, such as silver linden, passionflower, and honey.[13] For a while now, it has been used by bodybuilders and in some health situations, which include the management of anxiety, inflammatory diseases, gout, and erectile dysfunction.[13] Flavonoids belong to the main classes of polyphenols, which have numerous pharmacological activities and exert antioxidant effects. Chrysin has a structure that contains additional hydroxyl groups at the fifth and seventh positions of the A-ring, resulting in its potential to act as a free radical scavenger.[13,14]

This study explored the possibility of using chrysin as a nephroprotective agent against methotrexate-induced tubulointerstitial nephritis, its level of efficacy, and any possible adverse effects. The primary focus evaluated the potential of 5,7-dihydroxyflavone in attenuating oxidative stress within the renal tubules, renal interstitium, and renal parenchyma. Exploring the efficacy of 5,7-dihydroxyflavone on methotrexate can be a game changer in the management of cancer patients.

Materials and Methods

Experimental animals

A total of 25 female Wistar rats of an average weight of 200 g were acquired from Jroyal Affairs Integrated Farms, 39 Obollo Road, Nsukka, Enugu State, and housed for 6 weeks (October – November, 2023) in a well-ventilated room within the animal house of the Department of Anatomy, Enugu State University College of Medicine (ESUCOM). The female Wistar rats were acclimated for two weeks before experimental manipulations commenced. They were fed with commercial growers' mash and water *ad libitum*. The design of the experiment is shown in Table 1 below. All procedures within this study were according to the Animal Care and Use Standard Operating Procedures and Guidelines (SOPGs).[15]

Ethical approval

Ethical clearance for experimental animal use in this study was granted by the Ethics and Research Committee of the Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, with the ethical right permission number: ESUCOM/FBMS/ETR/2023/027.

Drugs

Methotrexate tablets IP (2.5 mg) used in this study was procured from Healing Pharma India Pvt. ltd, B-411, Western Edge II Premises CHS, Western Express Highway, Borivali (East), Mumbai. The phytochemical pure substance, chrysin (5, 7-dihydroxyflavone), was procured from Sigma-Aldrich Company, 3050 Spruce Street, St. Louis, USA.

Experimental design

The study design consisted of 25 adult female Wistar rats randomly separated into five groups consisting of five rats each (A-E, n = 5). The dosage and administration of methotrexate were according to the methods described by Sen *et al.* (2014)[16], while the dosage and administration of chrysin were according to Egwuatu *et al.* (2023)[14].

Serum kidney function/damage assay

On day 29, before the euthanization of experimental animals, blood samples were obtained via the retro-orbital venous plexus using a capillary tube. The samples collected were immediately centrifuged at 4,000 rpm at 4˚C for 10 minutes. The serum obtained from the samples was stored at -80˚C and electrolytes (sodium, potassium, bicarbonate, and chloride), blood urea nitrogen (BUN), and serum creatinine were assayed for kidney damage using commercially available Roche and Cobas test kits.[17]

Oxidative stress assay

The animals were euthanized using chloroform, and their kidneys were quickly excised and cleaned with ice-cold saline. The right kidneys were frozen in liquid nitrogen and stored at -80˚C until the analysis, while the left kidneys were fixed in a 10% neutral buffered formalin (NBF) solution for 48 hours and used for histopathological examination. The homogenization procedure was performed under standardized conditions to extract the supernatant of the kidney homogenate in Tris-ethylenediaminetetraacetic acid (EDTA) buffer for superoxide dismutase (SOD)[18], glutathione peroxidase (GPx)[19], and catalase (CAT)[20] activities. Lipid peroxidation was quantified using malondialdehyde (MDA) according to Satyam.[21]

Histopathology analysis

The kidney tissues were processed, and the paraffin blocks obtained were sliced into 5-mm-thick sections. The sections were stained with hematoxylin and eosin (HE) for light microscopy (LM).[22] The sections were analyzed under the light microscope for vacuolar and degenerative alterations to the renal glomerulus, interstitium, and tubules.

Statistical analysis

Quantitative data (serum electrolytes, creatinine and BUN, SOD, GPx, CAT, MDA, and inflammatory markers) were described as mean and SD for various groups. One-way analysis of variance (ANOVA) was used to compare all groups, followed by Tukey's comparison test. Data were analyzed using the SPSS Version 26 (IBM Corp., Armonk, NY, USA) software.

Results and Discussion

Effect of chrysin on kidney function in methotrexate-induced TIN

The effects of chrysin on the serum electrolytes (Na^+ and K^+), serum creatinine (Cr), and blood urea nitrogen (BUN) are represented in Table 2. Compared to the normal control group (group A), group B (methotrexate-only group) significantly demonstrated a higher level of Na^+, K^+, Cr, and BUN ($p < 0.05$), suggesting that nephrotoxicity in the female Wistar rats was caused by methotrexate. Group D also revealed a significantly lower level of K^+ and higher levels of creatinine and BUN ($p < 0.05$).

Methotrexate-induced nephrotoxicity has been one of the most dreaded methotrexate complications in cancer management due to its high-dose requirement to be therapeutic when engaged in chemotherapy.[23] MTX is primarily excreted by the kidneys, and the first signs of MTX nephrotoxicity appear with a reduction in glomerular filtration rate, precipitation within the renal tubules, and subsequently, electrolyte disturbances such as Na^+ and K^+ wasting.[2424]

In this study, the serum electrolytes in the methotrexate-only group demonstrated higher levels of Na^+ and K^+ compared to the normal control group, as seen in Table 2 above, suggesting MTX-induced kidney failure in group B.[26,26] In group D, the hypokalemia observed in this treatment group may have been a result of a resolving acute kidney injury.[27] However, the Na^+ levels of group D and other treatment groups remained essentially normal. This also suggested an indication of the preservation of renal functions by ameliorating electrolyte disturbances caused by high-dose MTX administration.[2424]

The creatinine and BUN levels recorded were significantly higher in the methotrexate-only group than in the normal control group (group A), as demonstrated in Table 2, again demonstrating the possibility of kidney damage induced by MTX.[2626] There was a slight elevation of creatinine and BUN in the low-dose treatment group, though they were significantly higher than the observed levels in the normal control group. This also suggested kidney damage and the inability of chrysin to attenuate the effects of methotrexate on the kidney glomerulus and tubulointerstitium in low doses. However, with high-dose chrysin doses, renal clearance was optimal, thus mitigating the detrimental effects of methotrexate on renal parenchyma. Our result is in tandem with a previous study by Sparks *et al.* (2021)[2828] that chrysin has the potential to attenuate nephrotoxicity and improve eGFR in low doses. However, it did state that chrysin is efficacious in the management of methotrexate-induced kidney damage in humans. The disparity in dosage may be due to the disparity in the experimental model.

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Table 1: Experimental design and grouping of animals

	Group description	Treatment
A	Control	0.2 mL of normal saline orally for 28 days.
B	MTX only	20 mg/kg bw of Methotrexate on days 8 and 22.
C	CHR only	100 mg/kg bw of Chrysin daily for 28 days.
D	MTX + Low-dose CHR only	50 mg/kg bw of Chrysin daily for 28 days, then 20 mg/kg bw of Methotrexate on days 8 and 22.
E	MTX + High-dose CHR only	100 mg/kg bw of Chrysin daily for 28 days, then 20 mg/kg bw of Methotrexate on days 8 and 22.

MTX= methotrexate, CHR= chrysin, bw= body weight.

Table 2: Effect of chrysin on serum electrolytes, creatinine, and BUN

	Na^+ (mmol/L)	K^+(mmol/L)	Cr (µmol/L)	BUN (mg/dL)
A	142.20 ± 10.62	5.81 ± 0.73	33.19 ± 3.20	36.67 ± 1.87
B	184.04 ± 6.58[a]	9.52 ± 1.01[a]	99.56 ± 5.02[a]	106.67 ± 4.62[a]
C	139.71 ± 8.32	5.18 ± 0.62	33.18 ± 2.61	36.32 ± 3.26
D	127.87 ± 9.19	3.46 ± 1.68[b]	55.25 ± 6.22[a]	53.33 ± 1.53[a]
E	133.48 ± 7.48	5.48 ± 0.31	40.65 ± 3.84	39.05 ± 2.12

Values were expressed as Mean ± SD; [a]$p < 0.05$ demonstrated a significantly higher level compared to group A; [b]$p < 0.05$ demonstrated a significantly lower level compared to group A. A= control group, B= methotrexate only group, C= chrysin only group, D= methotrexate+ low-dose chrysin group, E= methotrexate+ high-dose chrysin group.

Effect of chrysin on oxidative stress enzyme activities of the kidney in methotrexate-induced TIN

Tubulointerstitial nephritis (TIN) is alleged to be an immune-mediated inflammatory disease that is commonly drug-induced.[2929] Methotrexate-induced TIN has been established to be associated with the induction of reactive oxygen species (ROS), which leads to oxidative stress in Wistar rats. The effects demonstrated in our study on kidney oxidative stress enzyme activities are summarized in Table 3. The pathophysiology of TIN when induced by methotrexate involves precipitation of metabolites within the renal interstitium and tubules, leading to inflammation and the release of inflammatory cytokines. These further cause collagen accumulation in the extracellular matrix and basement membrane, leading to fibrosis and the release of reactive oxygen species which results in oxidative stress-induced mitochondrial injury.[2929] Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase are well-known scavengers of these free radicals released by ROS. The activities of SOD and GPx in group B were markedly reduced, with a significant increase in the activity of MDA, demonstrating oxidative damage in the kidney tissues. Elevated MDA tissue activity is an indicator of lipid peroxidation, which may result from an increase in free oxygen radicals leading to tissue oxygen damage.[3030] The demonstration of significantly reduced activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the methotrexate-only group in comparison to the normal control group as seen in Table 3 above may suggest ongoing renal oxidative stress and possibly tubular injury. On the contrary, the high-dose chrysin treatment group demonstrated insignificant activities of SOD and GPx in kidney tissues in comparison to the normal control group ($p < 0.05$). In group D, there was a significantly lesser level of SOD activity in comparison to the normal control group, with MDA, CAT, and GPx demonstrating insignificant differences compared to the normal control.

This suggested moderate protection of the renal parenchyma against oxidative stress due to high-dose methotrexate administration, similar to Sparks *et al*.'s report (2021)[2828]. In addition, there was no significant difference in the activities of CAT between the normal control group (group A) and the chrysin-treated groups. Still, the same could not be said for the methotrexate-only group, which demonstrated markedly reduced levels of CAT ($p < 0.05$).

In group E, as shown in Table 3, the mean activity levels of oxidative stress markers (SOD and GPx) and lipid oxidation (MDA) were not significantly different in comparison to the normal control group. This showed that a high dosage of chrysin protected against high-dose methotrexate-induced TIN. The mean level of catalase (CAT) was also markedly reduced in group B (methotrexate-only) group, thus suggesting oxidative stress; however, the same was not obtained for the treatment group, suggesting attenuation of oxidative stress by chrysin on the kidney parenchyma.[2020] In this study, our results propose that the oxidative stress markers (SOD,[3131] GPx, and CAT) and lipid peroxidation (MDA) are improved with the administration of chrysin in the management of methotrexate-induced tubulointerstitial nephritis, implying that chrysin has the potential to restore and maintain the level of enzymatic activities in methotrexate-injured kidneys.

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Effect of chrysin on the histology of the kidney in methotrexate-induced TIN

The photomicrographs from the histopathological examination provided strong backing to the biochemical results. Figures 1A and 1C show the normal control group (group A) and group C, where the photomicrographs of the kidney showed normal glomerulus (black arrows), tubules, and interstitium. Histopathological analysis of tubulointerstitial nephritis is usually characterized by interstitial inflammation with evident hydropic changes and marked tubular dilatation.[32] These were evident in the methotrexate-only group (group B), as shown in Figure 1 above. The photomicrograph of group B demonstrated severe glomerular necrosis, tubular necrosis, and

Table 3: Effect of chrysin on oxidative stress markers of the kidney

	SOD (U/mL)	GPx (mM)	CAT (U/mL)	MDA (µM)
A	1.33 ± 0.11	1.24 ± 0.16	32.50 ± 5.21	1.24 ± 0.71
B	0.74 ± 0.04[b]	0.26 ± 0.01[b]	4.88 ± 0.34[b]	3.90 ± 1.63[a]
C	1.27 ± 0.03	1.02 ± 0.07	17.88 ± 8.12	1.66 ± 0.28
D	0.85 ± 0.08[b]	0.97 ± 0.18	21.38 ± 6.97	1.19 ± 0.66
E	1.24 ± 0.10	1.14 ± 0.29	23.00 ± 4.42	1.70 ± 0.84

Values were expressed as Mean ± SD; [a]$p < 0.05$ demonstrated a significantly higher level compared to group A; [b]$p < 0.05$ demonstrated a significantly lower level compared to group B. A= control group, B= methotrexate only group, C= chrysin only group, D= methotrexate+ low-dose chrysin group, E= methotrexate+ high-dose chrysin group.

changes, which suggested progressive tubulointerstitial necrosis within the renal parenchyma of the Wistar rats in the group. The photomicrographs of the methotrexate-only group (group B) showed severe glomerular necrosis (red arrows) and an area of tubular necrosis (yellow arrows), with moderate edematous changes [Figure 1B]. Group D (low-dose treatment group) showed moderate glomerular necrosis (red arrows), but an essentially normal tubulointerstitial appearance with mild hydropic changes was noted (black arrow) [Figure 1D]. Group E showed lower levels of tubular dilation. The photomicrographs in group E were characterized by normal glomerulus and tubulointerstitium [Figure 1E].

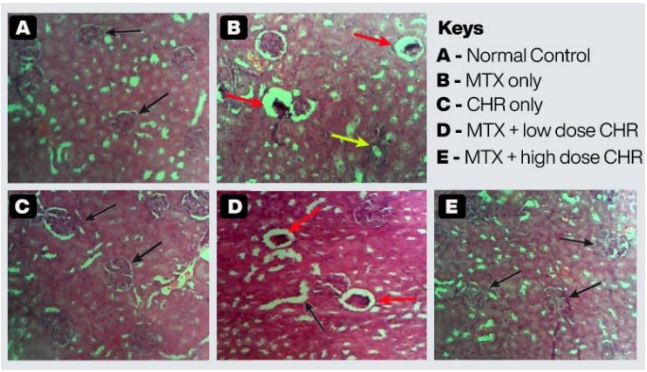

Figure 1: Photomicrograph of kidney hematoxylin-eosin dye sections from rats in groups A to E (X200). A displayed normal glomeruli (black arrows), tubules, and interstitium. B displayed severe glomerular necrosis, thickened basement membrane with moderate edematous changes indicated by the red arrows and area of tubular necrosis (yellow arrow). C displayed normal glomeruli, tubules and interstitium. D displayed moderate glomerular necrosis (red arrows) with mild hydropic changes. E displayed normal glomeruli (red arrow) with mild tubular dilation. [A= control group, B= methotrexate only group, C= chrysin only group, D= methotrexate+ low-dose chrysin group, E= methotrexate+ high-dose chrysin group].

The photomicrograph in group D also demonstrated moderate glomerular necrosis but an essentially normal tubulointerstitial appearance with mild hydropic changes. This proposes that chrysin administration in low dosage attempted to mount protection or restore the renal parenchyma but was overwhelmed. A different observation was noted in the photomicrograph of the high-dose treatment group (group E), characterized by a lower level of tubular dilatation but essentially with a normal glomerulus and interstitium. When compared to the normal control, group C demonstrated normal renal parenchyma. These observations supported the biochemical results obtained, as the evidence of tubular damage by methotrexate treatment was attenuated by high-dose chrysin. Again, our study suggests that the use of chrysin during chemotherapy would serve to attenuate the side effects of methotrexate on kidney tissues. Yet, the mechanism of action of chrysin on the renal parenchyma in the attenuation of methotrexate-induced tubulointerstitial nephritis was not determined.

Conclusion

In the current study, we have established that chrysin successfully precludes methotrexate-evoked tubulointerstitial nephritis. This is the first study to report significant attenuation of methotrexate-induced tubulointerstitial nephritis by chrysin. The impact of chrysin on renal dysfunction caused by methotrexate was assessed through histological analysis of kidney tissues, measuring serum levels of urea, creatinine, and electrolytes, as well as results measuring serum levels of oxidative stress enzyme activities. Based on the results demonstrated, it can be deduced that chrysin provided nephroprotective effects in high-dose methotrexate-administered Wistar rats. Thus, it can be comfortably proposed that chrysin has a potential nephroprotective role against methotrexate-induced tubulointerstitial nephritis. Nevertheless, further studies are recommended to evaluate the drug-drug interaction between chrysin and methotrexate.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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