Journal of Pharmacy & Allied Medicine

JPAM, 3(2), 89-96, 2025
https://doi.org/10.58985/jpam.2025.v03i02.35
ISSN (Print): 3005-2726

Research Article

Protective potential of 5-7, dihydroxyflavone (chrysin) on jejunum of adult female Wistar rats with methotrexate-induced toxicity

Kenechukwu Emmanuel Nwanama[1], Ikenna Kingsley Uchendu[2*], Juliet Chioma Iteshi[2], Martin Chukwuemeka Nnaji[2], Ndukwe Chika Kalu[3], Chiadikaobi Chrisantus Anih[4], Ejim Darlington Chukwuebuka[2], Chinwike Emmanuel Nnanna[5], Ochiaka Johnpaul Chukwunonso[6], Maryrose Chikaosolu Okafor[7], Ummusalma Abdulraheem[8], Henry Odey Ituma[9], Chidimma Sandra Ngwu[10], Chidiebere Emmanuel Nwafor[2] and **Williams Esther Ubong[11]**

1. Anatomy Unit, Sancta Maria Catholic College of Nursing Sciences, Uzairue, Edo State, Nigeria.
2. Departtment of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria.
3. Erismann Institute of Public Health, I.M Sechenov First Moscow State Medical University, Moscow, Russia.
4. Department of Medical Laboratory Science, Enugu State University of Science and Technology, Enugu State, Nigeria.
5. Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria.
6. Department of Biochemistry, University of Nigeria, Nsukka Campus, Enugu State, Nigeria.
7. Department of Biotechnology, I.M Sechenov First Moscow State Medical University, Moscow, Russia.
8. Science Department, Kuntau Science Academy, Kano, Kano State, Nigeria.
9. Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
10. Department of Medical Laboratory Science Madonna University, Elele Campus, Rivers State, Nigeria.
11. Department of Medical Laboratory Science, University of Calabar, Calabar, Cross River State, Nigeria.

Article Information
Received: 24 May 2025
Revised: 16 June 2025
Accepted: 18 June 2025
Published: 27 June 2025

Academic Editor
Prof. Dr. Christian Celia

Corresponding Author
Prof. Dr. Ikenna Kingsley Uchendu
E-mail: Ikenna.uchendu@unn.edu.ng
Tell: +2347068199556

Keywords
Chrysin, 5-7-dihydroxyflavone, methotrexate, jejunal toxicity, inflammation, oxidative stress.

Abstract

The jejunum is delicate because of its nutrient absorptive and immune functions. This study aimed to investigate the potential protective effect of chrysin on methotrexate-induced jejunal toxicity using adult female Wistar rats. This experiment used a total of 24 rats, randomly divided into 6 groups. Group I, served as the normal control and received food and water. Group II, served as a negative control and was induced with methotrexate only (20 mg/kg), Group III received low dose of chrysin (50 mg/kg), Group IV was induced with methotrexate (20 mg/kg) and a low dose of chrysin (50 mg/kg), Group V received a high dose of chrysin (100 mg/kg) and Group VI induced with methotrexate (20 mg/kg) and a high dose of chrysin (100 mg/kg) respectively. At the end of the experiment, jejunal toxicity was measured biochemically and histo-pathologically by light microscopy, and body weight was statistically analyzed using the SPSS version 23 package. Methotrexate elevated serum MDA in the methotrexate only group (1.24 ± 0.71) compared to the control group (1.14 ± 0.32) and reduced antioxidant enzymes (SOD, GSH, CAT), indicating oxidative stress ($p < 0.05$). Serum TNF-α, IL-1β, IL-6, and PGE-2 levels showed an increase across groups induced with methotrexate, indicating inflammation. Chrysin treatment significantly reduced these biomarkers compared to the control group ($p < 0.05$). Histological analysis showed methotrexate caused jejunal necrosis, while chrysin alone or with methotrexate preserved normal histoarchitecture. Low and high doses of chrysin improved tissue structure, though high doses alone showed metaplasia. Chrysin is a potential therapeutic agent against methotrexate-induced jejunal toxicity, reducing oxidative damage and inflammation.

1. Introduction

Methotrexate (MTX) is one of the medications commonly prescribed for cancer, particularly in the management of leukemia, lymphoma, and breast cancer [1]. It is also employed in the management of autoimmune diseases such as rheumatoid arthritis and psoriasis [2]. Its mechanism of action is to prevent the function of dihydrofolate reductase (DHFR) [3]. This inhibition prevents the division of rapidly dividing cells which is a characteristic of cancerous cells. However, its use is restricted due to its toxicity to the gastrointestinal tract (GI), especially the small intestine (jejunum) [4]. GI toxicity of the drug is most apparent in the jejunum, during nutrient absorption [4, 5]. MTX also induces malnutrition via malabsorption of nutrients from the jejunum due to its actions on the villi and mucosal tissues [5, 6]. Patients undergoing chemotherapy with high-dose MTX at times come down with mucositis and/or enteritis, resulting from severe jejunal damage. This manifests clinically with symptoms such as nausea, vomiting, diarrhea, and malabsorption [4, 7]. These side effects reduce patient quality of life and the use of MTX in clinical practice is usually under professional monitoring [8].

MTX causes jejunal toxicity through several pathways such as oxidative stress, inflammation, and apoptosis [5, 6]. Oxidative stress caused by MTX use is attributed to the release and accumulation of free radicals, such as reactive oxygen species, which have detrimental effects on lipids, proteins, and DNA of the cells [9]. Overall, the increase in oxidative stress and inflammation leads to injury and destruction of the jejunal epithelial cells, thereby affecting the integrity of the jejunal mucosa and its function [4].

Chrysin (5,7-dihydroxyflavone) is a flavonoid that naturally occurs in honey, propolis, and other plants [10]. It can serve as an antioxidant, which can be employed to protect against the negative effects of methotrexate chemotherapy [11]. Chrysin also possesses anti-inflammatory and anti-apoptotic effects, and this substance may help reduce the toxicity of MTX through antagonistic actions [12]. Flavonoids are natural polyphenolic compounds that are present in many fresh fruits, vegetables, and some drinks such as tea and wine [13]. As reported by Singh and Verma [14], flavonoids present in these beverages have antioxidant activity, reduce inflammation, and possess anti-cancer effects. Some evidence has revealed that chrysin can directly remove free radicals and also increase the level of the cellular antioxidant enzymes SOD and catalase and thus decrease oxidative stress [15]. These activities can be explained through the suppression of pro-inflammatory cytokines and enzymes [16]. Moreover, chrysin has been found to affect apoptotic pathways and minimize the extent of cell death as well as enhance cell survival in various tissues [17]. Although chrysin has the properties described above, there are few studies focused on the protective role of chrysin against MTX-induced jejunal toxicity, such as those reported by Sirichoat *et al*. [11], that evaluated the protective effects of flavonoids, including chrysin, against intestinal (jejunal) damage caused by various drugs, with implications for MTX treatment.

MTX-induced jejunal toxicity is a significant clinical problem, as it can lead to severe morbidity and mortality [4]. The exact mechanisms of MTX-induced jejunal toxicity are not fully understood but are thought to involve the inhibition of DNA synthesis and cell division in the rapidly dividing cells of the intestinal mucosa [18]. Currently, there are limited treatment options for preventing or treating MTX-induced gastrointestinal toxicity [19]. Supportive care, such as anti-diarrheal medications and nutritional supplements, is often inadequate, and there is a need to explore alternative strategies for mitigating this toxicity [7]. The aim of this study was to investigate the potential protective effect of chrysin in methotrexate-induced jejunal toxicity using adult female Wistar rats.

2. Materials and methods

2.1. Procurement of chemical and inducing agent

The chrysin (5,7-dihydroxyflavone) was gotten from Sigma Aldrich company, St Louis, USA and the methotrexate was gotten from Healing Pharmacy LLC, USA.

2.2. Experimental animal

In the experiment, 24 female adult albino Wistar rats (120–200 g) were purchased and housed in the animal laboratory room at Sancta Maria Catholic College of Nursing Sciences Uzairue. The rats were acclimatized

https://doi.org/10.58985/jpam.2025.v03i02.35

for 3 weeks prior to experimental use. They were housed in a well-ventilated room at normal room temperature and in conducive conditions throughout the experiment. Animals were fed with certified livestock pellet feed and clean water.

2.3. Experimental design

The animals were randomly divided into six groups and treated as follows: Group I, served as the normal group; they were fed with food and water. Group II, served as a negative control and was induced with methotrexate only (20 mg/kg), Group III with a low dose of chrysin (50 mg/kg), Group IV was induced with methotrexate (20 mg/kg) and a low dose of chrysin (50 mg/kg), Group V received a high dose of chrysin (100mg/kg) and Group VI induced with methotrexate (20mg/kg) and a high dose of chrysin (100 mg/kg) respectively (Table 1). All the administration was done orally. methotrexate was induced daily for 8 days, while chrysin administration lasted for 28 days.

2.4. Sacrifice of experimental animal

Twenty-four hours after the last administration for various groups, the rats were weighed and sacrificed by cervical dislocation. A mild incision was made through the body. The small intestine (jejunum) is then removed and fixed for histological investigations. Blood was collected via retro-orbital sinus bleeding using a capillary tube, and clotting was allowed for about 2 hours. Thereafter, the clotted blood was centrifuged for 10 minutes to recover serum from the blood cell. The serum was separated from clotted blood for biochemical analysis.

2.5. Histological procedure

The small intestine (jejunum) was subjected to histological studies, in which tissues from all groups were fixed in 10% formalin solution, and tissue was processed using a paraffin wax embedding medium. Tissue blocks were microtome using a rotator microtome and sections stained with routine H&E stain for histological examination.

2.6. Blood sample collection and serum preparation

Blood samples were collected from all groups through the retro-orbital sinus, using a capillary tube. The blood samples were spined using an 800D centrifuge machine, after which the serum was separated from the blood cells. The serum is then collected and taken for biochemical analysis of inflammatory and oxidative stress markers.

2.7. Statistical analysis

Data were analyzed using a statistical package for social sciences (SPSS) version 23.0. Data obtained was presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to check for differences between means, which was considered significant at $p \leq 0.05$ following post-hoc test (Tukey).

3. Results and discussion

Findings from the malondialdehyde (MDA) levels amongst all the groups indicated that animals in Group B which were given 20 mg/kg of methotrexate, only showed mean elevated serum MDA levels (3.90 ± 1.63) when compared to the treatment Groups 1 and 3, which had lower mean serum MDA levels (1.24 ± 0.71 and 1.14 ± 0.32, respectively). However, the mean serum MDA levels of animal groups that received chrysin treatment following methotrexate exposure were restored to normal (Table 2). The Tukey post hoc test revealed that the mean serum level of MDA for animals in Group 2 was significantly ($p < 0.05$) higher than the mean level of MDA of animals in Group 1. The mean SOD, GSH and CAT levels of animals in Group 2 was significantly lower than the mean levels of animals in Group 1. In the treatment groups, following the administration of chrysin after the induction of methotrexate, the levels of SOD, GSH and CAT were elevated especially in Group 4 and 6 which received 20 mg/kg of methotrexate and chrysin (50 and 100 mg/kg, respectively) treatment. Also, the administration of chrysin decreased the level of MDA in the treatment group. Thus, this study showed that chrysin inhibits high MDA levels in methotrexate-induced oxidative stress. High levels of malondialdehyde (MDA) in serum are typically indicative of increased oxidative stress in the body. Reactive Oxygen Species (ROS) is one of the most important reasons in the mechanism of damage caused by anticancer agents in kidney tissue [20]. The formation of uncontrollable ROS causes lipid peroxidation in the cell membrane structure, oxidation of proteins and enzymes [21]. Tissue MDA level is an important and reliable marker of degradation in the oxidation of polyunsaturated fatty

https://doi.org/10.58985/jpam.2025.v03i02.35

Table 1. Experimental design

Groups	Number of rats	Dosages	Ttreatments
GROUP 1 Normal Control	4	Control Food and water only	Food pellet and distilled water only will be given for 28 days.
GROUP 2 Negative control (Methotrexate only)	4	Methotrexate (20mg/kg) only	20mg/kg of methotrexate was given daily for 8 days.
GROUP 3 (Chrysin- low dose)	4	Low dose of chrysin (50mg/kg)	50mg/kg of chrysin was administered for 28 days
GROUP 4 (Methotrexate + low dose chrysin)	4	Methotrexate (20mg/kg) and low dose of chrysin (50mg/kg)	20mg/kg of methotrexate was given for 8 days and treated with 50mg/kg of chrysin which was administered for 28 days.
GROUP 5 (Chrysin–high dose)	4	High dose of chrysin (100mg/kg)	100mg/kg of chrysin was administered for 28 days.
GROUP 6 (Methotrexate + high dose chrysin)	4	Methotrexate (20mg/kg) and high dose of chrysin (100mg/kg)	20mg/kg of Methotrexate was administered for 8 days and treated with 100mg/kg of chrysin which was given for 28 days.

Table 2. Effect of chrysin on anti-oxidative stress markers

Group (n-4)	SOD (U/ml)	GSH (µmol/mL)	CAT (U/ml)	MDA (nmol/mL)
GROUP 1 Normal control	1.33 ± 0.11	1.24 ± 0.16	32.50 ± 5.21	1.24 ± 0.71
GROUP 2 Negative control (Methotrexate only)	0.74 ± 0.04[b]	0.26 ± 0.01[b]	4.88 ± 0.34[b]	3.90 ± 1.63[a]
GROUP 3 (Chrysin- low dose)	1.13 ± 0.01	1.17 ± 0.24	22.75 ± 3.58	1.14 ± 0.32
GROUP 4 (Methotrexate + low dose chrysin)	1.27 ± 0.03	1.02 ± 0.07	17.88 ± 8.12	1.66 ± 0.28
GROUP 5 (Chrysin–high dose)	0.85 ± 0.08[b]	0.97 ± 0.18	21.38 ± 6.97	1.19 ± 0.66
GROUP 6 (Methotrexate + high dose chrysin)	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 1; [b]$p < 0.05$ (SOD, GSH, and CAT) demonstrated a significantly lower level compared to Group 2. [b]$p < 0.05$ (MDA) demonstrated a significantly higher level compared to Group 1.

acids. Chrysin has a pronounced protective effect against lipid peroxidation and reduced renal MDA production. Our results are in line with several reports of increased MDA levels in tissues due to methotrexate-induced oxidative stress. Chrysin's ability to scavenge free radicals significantly contributes to its inhibitory effect on lipid peroxidation. SOD antioxidant enzyme, which is directly responsible for the detoxification of ROS, is present in high concentration in intestinal tissue. Methotrexate treatment in this study significantly reduced levels of SOD according to current published studies. Considering its antioxidant activity, chrysin used in the treatment was similarly able to prevent the decrease in SOD activities in the rat jejunum tissue [22]. This effect may be due to an improvement in antioxidant status and scavenging of excess free radicals such as O^{2-} and peroxyl radicals. Similar

https://doi.org/10.58985/jpam.2025.v03i02.35

Table 3. Effect of chrysin on inflammatory markers

Group (n-4)	IL-1β (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)	PGE-2 (pg/ml)
GROUP 1 Normal control	31.0 ± 0.6	42.6 ± 2.1	55.6 ± 2.4	218.1 ± 3.0
GROUP 2 Negative control (Methotrexate only)	35.8 ± 2.3	261.9 ± 5.4	72.5 ± 3.7	95.3 ± 5.0
GROUP 3 (Chrysin- low dose)	31.6 ± 8.2	66.8 ± 3.4	61.2 ± 2.0	239.1 ± 2.0
GROUP 4 (Methotrexate + low dose chrysin)	33.7 ± 3.4	51.9 ± 3.0	56.1 ± 0.9	299.2 ± 3.0
GROUP 5 (Chrysin–high dose)	35.1 ± 2.2	189.0 ± 5.3	65.2 ± 0.8	119.7 ± 2.0
GROUP 6 (Methotrexate + high dose chrysin)	29.0 ± 0.5	48.2 ± 2.1	59.2 ± 7.5	201.2 ± 1.0

results have been obtained with different antioxidants such as melatonin and proanthocyanidin, which structurally protect antioxidant enzymes and increase their activity in methotrexate -induced nephropathy [16].

Methotrexate's effect on inflammatory markers was examined in this study. Findings from the TNF-α levels amongst all the groups indicated that animals in Group 2, which were given 20mg/kg of methotrexate only, showed mean elevated serum TNF-α levels (261.9 ± 5.4) when compared to the control and chrysin Groups 1 and 3, which had lower mean serum MDA levels (42.6 ± 2.1 and 66.8 ± 3.4, respectively). However, the mean serum TNF-α levels of animals' groups that received chrysin treatment following methotrexate exposure were restored to be comparable to normal (Table 3). The mean IL-1β, IL-6 and PGE-2 levels of animals in Group 2 were significantly higher than the mean levels of animals in Group 1 and other groups which received chrysin. Thus, this study showed that chrysin inhibits high levels of methotrexate-induced inflammation. The inflammatory pathway depending on the TNF-α transcription is an important mechanism which has been previously reported to be involved in the methotrexate-induced inflammatory response by increasing the interleukins expression [23]. In our study, methotrexate mediated the inflammatory response in the jejunum through elevation of the protein levels of TNF-a, IL-1β, IL-6 and PGE-2. While chrysin treated groups revealed significant decreases in the methotrexate-induced elevated inflammatory markers, as shown in Table 3. Chrysin, a natural flavonoid, demonstrates protective effects against methotrexate (MTX)-induced toxicity in various models of intestinal inflammation. These findings agreed with previous studies which concluded that the chrysin treatment reduced the inflammatory response via lowering the pro-inflammatory cytokines in jejunum tissue. Previous studies showed that chrysin and its derivatives reduced pro-inflammatory cytokine levels, including TNF-α, IL-6, and MCP-1, in both in vitro and in vivo experiments [24, 25]. The anti-inflammatory action of chrysin is mediated through the inhibition of NF-κB signaling pathways in vascular endothelial cells [26], and suppression of JAK-STAT signaling in macrophages [25]. Additionally, chrysin exhibits antioxidant properties by scavenging reactive oxygen species, which act as upstream mediators of inflammatory signaling [25]. In colorectal cancer models, chrysin treatment significantly reduced levels of pro-inflammatory factors such as amphiregulin, CXCL1, and MMP-9, while also demonstrating anticancer activity against SW620 cells by decreasing ERK and AKT phosphorylation [27]. These findings suggest that chrysin may be a promising therapeutic candidate for inflammatory bowel diseases. Hence, our data clarified that chrysin shows anti-inflammatory activity against the MTX-induced inflammatory response.

The histological features of the jejunum in our present study were analyzed to further study the histoarchitecture of the tissue (Fig. 1).

https://doi.org/10.58985/jpam.2025.v03i02.35

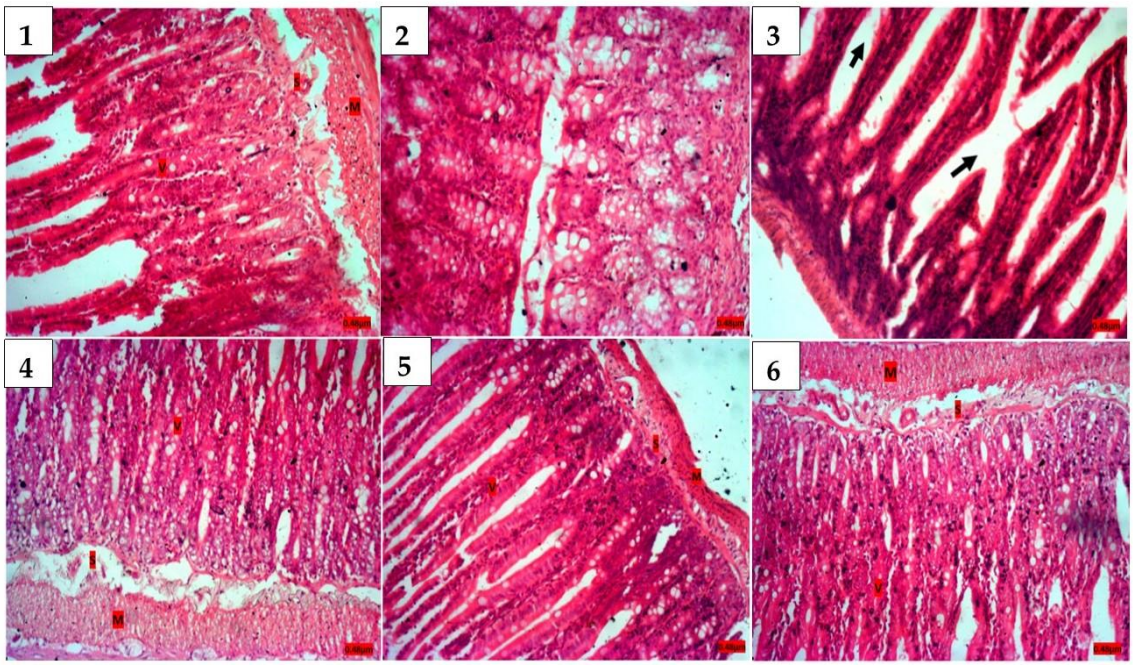

Figure 1. Group 1: Photomicrograph of the jejunum microstructure showing intestinal villi (V), submucosa (S) and the muscularis externa layer (M). Tissue appears normal. Group 2: Photomicrograph of the jejunum microstructure showing intestinal villi (V), submucosa (S) and the muscularis externa layer (M). Tissue appears normal. Group 3: Photomicrograph of the jejunum microstructure showing inter-villous space necrosis (arrow). Group 4: Photomicrograph of the jejunum microstructure showing intestinal villi (V), submucosa (S) and the muscularis externa layer (M). Tissue appears normal. Group 5: Photomicrograph of the jejunum microstructure showing general tissue metaplasia Group 6: Photomicrograph of the jejunum microstructure showing intestinal villi (V) and the muscularis externa layer (M). Tissue appears normal. H & E. X300

The control group photomicrograph of the jejunum microstructure shows normal intestinal villi. The negative control induced with methotrexate presents inter-villous space necrosis, while groups 4 and 6, treated with MXT and chrysin, show normal intestinal villi. However, Group 5, which received a high dose of chrysin (100 mg/kg) only, the photomicrograph of the jejunum tissue microstructure shows general tissue metaplasia. Methotrexate (MTX) can cause significant intestinal damage, including villus shortening, crypt damage, inflammation, and oxidative stress [28, 29]. MTX-induced intestinal toxicity is associated with alterations in gut microbiota, particularly a decrease in *Bacteroides fragilis*, and changes in immune response, such as increased macrophage density [30]. Several studies have explored protective interventions against MTX-induced intestinal injury. In rats exposed to propetamphos, chrysin mitigated oxidative stress, improved biochemical parameters, and reduced histopathological damage [31].

These studies collectively demonstrate chrysin's potential as a protective agent against various organ toxicities and diseases, likely due to its antioxidant properties and ability to modulate key biochemical parameters. The findings suggest that chrysin may have therapeutic potential in treating conditions such as organ toxicity, while chrysin showed significant antioxidant and anti-inflammatory protection in MTX-induced rats. Histological analysis of Group 5 (100 mg/kg chrysin alone) revealed metaplastic changes, suggesting dose-dependent toxicity. This may be attributed to chrysin's poor oral bioavailability and metabolism, as previously reported [32]. Without absorption enhancers, systemic exposure is limited, potentially causing tissue-specific accumulation. Compared to standard protectants like folic acid, which is routinely co-administered with MTX to reduce toxicity, chrysin's safety profile requires further clarification. Moreover,

https://doi.org/10.58985/jpam.2025.v03i02.35

species differences in metabolism and gut microbiota between rats and humans must be considered before clinical translation, given chrysin's limited bioavailability in humans.

4. Conclusions

This study shows that 5,7-dydroxyflavone at a dose of 50 mg/kg body weight has a protective effect in the treatment of methotrexate-induced jejunum toxicity in adult female Wistar rats. These findings suggest that chrysin, with its antioxidant and anti-inflammatory properties, could serve as a potential therapeutic agent for protecting against methotrexate-induced intestinal toxicity, reducing oxidative damage, and inflammation.

Ethical Statement

The study's experimental protocol and techniques were based on international Animal use and care principles. Ethical approval (SMCCN/CREC/2024/010) was duly obtained and the study followed the guidelines of the ethics committee on animal research of Sancta Maria Catholic College of Nursing Sciences, Uzairue, Edo State.

Authors' contributions

Conceptualization, methodology, investigation, project administration, funding acquisition, resources,writing—review & editing, supervision, K.E.N.; Data curation; formal analysis, software, validation, visualization, K.E.N., I.K.U., J.C.I., M.C.N., N.C.K., C.C.A., E.D.C., C.E.N., O.J.C., M.C.O., U.A., H.O.I., C.S.N., C.E.N., W. E.U.

Acknowledgements

The authors don't have anything to acknowledge.

Funding

This research project did not receive any external funding.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of interest

The authors declare no conflicts of interest regarding this manuscript.

References

1. Yang, V.; Gouveia, M.J.; Santos, J.; Koksch, B.; Amorim I, Gärtner F, Vale N. Breast cancer: insights in disease and influence of drug methotrexate. RSC Med. Chem. 2020, 11(6), 646-664. https://doi.org/10.1039/d0md00051e
2. Kumar, S.; Garg, N.K.; Jain, A.; Khopade, A.; Pandey, P.; Sawant, K.K. Nanocarriers mediated delivery of methotrexate is instrumental in treating auto-immune diseases and cancer. J. Drug Deliv. Sci. Technol. 2023, 88, 104969. https://doi.org/10.1016/j.jddst.2023.104969
3. Sramek, M.; Neradil, J.; Veselska, R. Much more than you expected: the non-DHFR-mediated effects of methotrexate. Biochim. Biophys. Acta-Gen. Subj. 2017, 1861(3), 499-1503. https://doi.org/10.1016/j.bbagen.2016.12.014
4. Ali, G.F.; Hassanein, E.H.; Mohamed, W.R. Molecular mechanisms underlying methotrexate-induced intestinal injury and protective strategies. Naunyn-Schmiedeb Arch. Pharmacol. 2024, 397(11), 8165-8188. https://doi.org/10.1007/s00210-024-03164-x
5. Burcu, B.; Kanter, M.; Orhon, Z.N.; Yarali, O.; Karabacak, R. Protective Effects of Vitamin E on Methotrexate-Induced Jejunal mucosal damage in rats. Anal. Quant. Cytopathol. Histopathol. 2016, 38(2), 87-94.
6. Pottmeyer, G.W.; Dalal, B.D. Severe malnutrition and methotrexate toxicity as an unusual cause of bone marrow aplasia and multi-system organ failure. Chest. 2023, 164(4 Suppl), A1941. http://dx.doi.org/10.1016/j.chest.2023.07.1333
7. Akbarali, H.I.; Muchhala, K.H.; Jessup, D.K.; Cheatham, S. Chemotherapy induced gastrointestinal toxicities. Adv. Cancer Res. 2022, 155, 131-166. https://doi.org/10.1016/bs.acr.2022.02.007
8. Makuch, R.; Chrościcka, A.; Gała, K.; Czajka, A.; Lenard, P.; Kucharski, A.; Michalska, S.; Pilarski, K.; Dewicka, M.; Wawrzyniak, A.M. Therapeutic applications and challenges of a widely used antifolate drug. Quality Sport. 2024, 16, 52214-52214. https://doi.org/10.12775/QS.2024.16.52214
9. Saka, S.; Aouacheri, O.J. The investigation of the oxidative stress-related parameters in high doses methotrexate-induced albino Wistar rats. J. Bioequiv. Availab. 2017, 9(2), 372-376. https://doi.org/10.4172/jbb.1000327
10. Mani, R.; Natesan, V. Chrysin: Sources, beneficial pharmacological activities, and molecular mechanism of action. Phytochem. 2018, 145, 187-196. https://doi.org/10.1016/j.phytochem.2017.09.016
11. Sirichoat, A.; Anosri, T.; Kaewngam, S.; Aranarochana, A.; Pannangrong, W.; Wigmore, P.; Welbat, J.U. Neuroprotective properties of chrysin on decreases of cell proliferation, immature neurons and neuronal cell

https://doi.org/10.58985/jpam.2025.v03i02.35

survival in the hippocampal dentate gyrus associated with cognition induced by methotrexate. Neurotoxicol. 2022, 92, 15-24. https://doi.org/10.1016/j.neuro.2022.06.010
12. Çomaklı, S.; Özdemir, S.; Güloğlu, M. Chrysin attenuates paclitaxel-induced hepatorenal toxicity in rats by suppressing oxidative damage, inflammation, and apoptosis. Life Sci. 2023, 332,122096. https://doi.org/10.1016/j.lfs.2023.122096
13. Kopustinskiene, D.M., Jakstas, V.; Savickas, A.; Bernatoniene, J. Flavonoids as anticancer agents. Nutrients. 2020, 12(2), 457. https://doi.org/10.3390/nu12020457
14. Singh, S.; Verma, R. Exploring the therapeutic potential of flavonoids in the management of cancer. Curr. Pharm. Biotechnol. 2025, 26(1), 17-47. https://doi.org/10.2174/0113892010297456240327062614
15. Samarghandian, S.; Azimi-Nezhad, M.; Samini, F.; Farkhondeh, T. Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats. Canadian J. Physiol. Pharmacol. 2016, 94(4), 388-393. https://doi.org/10.1139/cjpp-2014-0412
16. Kandemir, F.M.; Kucukler, S.; Eldutar, E.; Caglayan, C.; Gülçin, İ. Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: A multi-biomarker approach. Sci. Pharm. 2017, 85(1), 4. https://doi.org/10.3390/scipharm85010004
17. Raina, R.; Bhatt, R.; Hussain, A. Chrysin targets aberrant molecular signatures and pathways in carcinogenesis. World Acad. Sci. J. 2024, 6(5), 45. https://doi.org/10.3892/wasj.2024.260
18. El-Sheikh, A.A.; Morsy, M.A.; Hamouda, A.H. Protective mechanisms of thymoquinone on methotrexate-induced intestinal toxicity in rats. Pharmacog. Mag. 2016, 12(Suppl 1), S76. https://doi.org/10.4103/0973-1296.176106
19. Hamed, K.M.; Dighriri, I.M., Baomar, A.F.; Alharthy, B.T.; Alenazi, F.E.; Alali, G.H.; Alenazy, R.H.; Alhumaidi, N.T.; Alhulayfi, D.H.; Alotaibi, Y.B.; Alhumaidan, S.S. Overview of methotrexate toxicity: A comprehensive literature review. Cureus. 2022, 14(9), e29518. https://doi.org/10.7759/cureus.29518
20. Sahoo, B.M.; Banik, B.K.; Borah, P.; Jain, A. Reactive oxygen species (ROS): key components in cancer therapies. Curr. Med. Chem. Anti-Cancer. Agents. 2022, 22(2), 215-222. https://doi.org/10.2174/1871520621666210608095512
21. Huang, G.; Zhang, Q.; Xu, C.; Chen, L.; Zhang, H. Mechanism of kidney injury induced by cisplatin. Toxicol. Res. 2022, 11(3), 385-390. https://doi.org/10.1093/toxres/tfac019
22. Baykalir, B.G.; Arslan, A.S.; Mutlu, S.I.; Ak, T.P.; Seven, I.; Seven, P.T.; Yaman, M.; Gul, H.F. The protective effect of chrysin against carbon tetrachloride-induced kidney and liver tissue damage in rats. Int. J. Vit. Nutr. Res. 2021, 91(5–6), 427–438. https://doi.org/10.1024/0300-9831/a000653
23. Katturajan, R.; Vijayalakshmi, S.; Rasool, M.; Prince, S.E. Molecular toxicity of methotrexate in rheumatoid arthritis treatment: A novel perspective and therapeutic implications. Toxicol. 2021, 461, 152909. https://doi.org/10.1016/j.tox.2021.152909
24. Song, H.Y.; Kim, W.S.; Kim, J.M.; Bak, D.H.; Han, J.M.; Lim, S.T.; Byun, E.B. A hydroxyethyl derivative of chrysin exhibits anti-inflammatory activity in dendritic cells and protective effects against dextran sodium salt-induced colitis in mice. Int. Immunopharmacol. 2019, 77, 105958. https://doi.org/10.1016/j.intimp.2019.105958
25. Qi, S.M.; Li, Q.; Jiang, Q.; Qi, Z.L.; Zhang, Y. Chrysin inhibits lipopolysaccharide-induced inflammatory responses of macrophages via JAK-STATs signaling pathway. Nan Fang yi ke da xue xue bao= Journal of Southern Medical University. 2018, 38(3), 243-250. https://doi.org/10.3969/j.issn.1673-4254.2018.03.02
26. Zhao, S.; Liang, M.; Wang, Y.; Hu, J.; Zhong, Y.; Li, J.; Huang, K.; Li, Y. Chrysin suppresses vascular endothelial inflammation via inhibiting the NF-κB signaling pathway. J. Cardiovasc. Pharmacol. Ther. 2019, 24(3), 278-287. https://doi.org/10.1177/1074248418810
27. Salama, A.A.; Allam, R.M. Promising targets of chrysin and daidzein in colorectal cancer: Amphiregulin, CXCL1, and MMP-9. Eur. J. Pharmacol. 2021, 892, 173763. https://doi.org/10.1016/j.ejphar.2020.173763
28. Karale, S.; Kamath, J.V. Naringin Attenuates methotrexate induced intestinal injury and oxidative stress in Wistar rats. Indian Drugs. 2017, 54(12), 58. https://doi.org/10.53879/id.54.12.11153
29. Shaoul, R.; Moati, D.; Schwartz, B.; Pollak, Y.; Sukhotnik, I. Effect of pomegranate juice on intestinal recovery following methotrexate-induced intestinal damage in a rat model. J. Am. Collg. Nutr. 2018, 37(5), 406-414. https://doi.org/10.1080/07315724.2017.1413961
30. Zhou, B.; Xia, X.; Wang, P.; Chen, S.; Yu, C.; Huang, R.; Zhang, R.; Wang, Y.; Lu, L.; Yuan, F.; Tian, Y. Induction and amelioration of methotrexate-induced gastrointestinal toxicity are related to immune response and gut microbiota. EBioMedicine. 2018, 33, 122-33. https://doi.org/10.1016/j.ebiom.2018.06.029
31. Tekeli, M.Y.; Çakır Bayram, L.; Eraslan, G.; Soyer Sarıca, Z. The protective effect of chrysin against oxidative stress and organ toxicity in rats exposed to propetamphos. Drug Chem. Toxicol. 2022, 45(6), 2664-2677.
32. Adangale, S.C.; Wairkar, S. Potential therapeutic activities and novel delivery systems of chrysin-a nature's boon. Food Bioscience. 2022, 1, 45, 101316.