Frontiers in Health Informatics www.healthinformaticsjournal.com
ISSN-Online: 2676-7104
2024; 13: 03 Open Access

Effects of Myricetin on Liver Functions Prior 5-Flurouracil in Male Rats

Farah Ahmed Hassan[1*], Nada Naji Al-Shawi[2]
[1*]Ministry of Health and Environment, AlZahra Hospital, Babylon, Iraq.
[2]University of Baghdad, College of Pharmacy, Department of Pharmacology and Toxicology, Baghdad, Iraq.
Email: [1*]farahahmed88af@gmail.com ,
nadaalshawi@copharm.uobaghdad.edu.iq

Abstract
5-Fluorouracil (5-FU) belongs to the chemotherapy class of anti-metabolites (anti-pyrimidine) is widely-used as an anticancer drug to treat solid cancers, such as those of the colon, breast, rectum, and pancreas. However, its clinical application is limited due to its gastrointestinal and hematological toxicity. Myricetin (3, 3′, 4′, 5, 5′,7-hexahydroxyflavone) is a naturally-occurring flavanol found in fruits, vegetables, teas, and medicinal plants that has antimicrobial, antiviral, anti-aging, and anti-diabetic activities. The goal of this study was to evaluate the effect of two doses of myricetin on 5-flurouracil-induced hepatotoxicity in Wistar rats. Forty-two male Wistar rats were divided into six groups of seven rats each: Control (group 1) rats received distilled water daily for 20 days; group 2 rats were intraperitoneally (IP)-injected with a single toxic dose (150 mg/kg) of 5-FU on day 20; groups 3 and 4 rats orally-received either 25 or 50 mg/kg/day of myricetin, respectively, for 20 days. Group 5 and 6 rats orally-received 25 or 50 mg/kg/day of myricetin, respectively, for 20 days, and were IP-injected with 5-FU on day 20. From the results obtained from this study, it can be concluded that myricetin increases antioxidant levels and reduces liver inflammation, decreasing negative side-effects. The inclusion of myricetin with 5-FU resulted in positive histological changes that enhanced liver function.

Keywords: 5-fluorouracil, Hepatotoxicity, Wistar rats, western blot analysis, Liver function.

Statements and Declarations:
Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.
Data availability declaration: All data supporting the findings of this study are available within the paper and its supplementary Information.
Funding declaration: No funds, grants, or other support was received.

1. Introduction

The 5- Fluorouracil Antimetabolites are chemotherapeutic drugs that bind to macromolecules like DNA and RNA, and interfere with vital metabolic procedures. This group of drugs constitute a class of substances that interfere with the normal functioning of cells (Al-hoshary and Zalzala 2023; Dakhel and Mohammed 2011). One drug within this group, 5-FU, inhibits uracil metabolism, and has become increasingly popular since its development in the 1950s (Longley, Harkin, and Johnston 2003; Tanaka et al. 2000). It targets difficult cancers like colorectal and pancreatic ductal adenocarcinoma, as well as malignancies that develop in organs like the cervix and breast. It is widely employed in the treatment of solid tumors, especially in the gastrointestinal system (Chalabi-Dchar et al. 2021). Tumor growth depends on the production of thymine, so 5-FU works by converting uracil into 5-fluoro-2′-deoxyuridine monophosphate, which inhibits the production of the thymidylate synthase enzyme, thereby inhibiting the production of thymine. By reducing the quantity of thymidylate synthase enzyme, the levels of 2′-deoxythymidine-5′-monophosphate (dTMP) also decrease, which is essential for DNA repair and replication, and can lead to cell dysfunction and disorder (Bhattacharya 2022; Nija 2023; Shihab and Mohammed 2019; Chibber et al. 2011).
While chemotherapy drugs (including 5-FU) are important for the treatment of cancer cells and prolong the lives of individuals with cancer, they also lead to serious adverse effects on healthy organs due to their non-specific modes of action (Schwab et al. 2008). In particular, 5-FU has the potential to cause adverse reactions such as myelotoxicity, gastrointestinal toxicity, and cardiotoxicity (Alter et al. 2006; Kimura and Okuda 1999), liver and kidney damage, and as 5-FU is broken down in the hepatic cells, it forms urea, fluoro-beta-alanine, dihydrouracil, carbon dioxide, and ammonia, causing hepatotoxicity and nephrotoxicity (Yousef and Aboelwafa 2017).
Various studies have investigated the use of natural ingredients to reduce the harmful effects of 5-FU (Burak and Imen 1999; Fideles et al. 2020; Jucá et al. 2020; Panche et al. 2016). The flavonoid myricetin (Myr) shows a wide range of beneficial health effects, including protecting the heart, reducing inflammation, and antidiabetic, anti-aging, antioxidative and hepatoprotective effects (Chua et al. 2011; Jiang et al. 2019; Lin et al. 2006; Navarro-Hortal et al. 2020; Yao et al. 2014).

Objectives: Investigate the possible protective effects of two different doses of myricetin, 25 and 50 mg/kg/day, on 5-FU-induced hepatotoxicity in rats.

2. Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies (Tveden-Nyborg et al. 2023).

2.1. Animals

Forty-two (42) male Wistar rats ranging between (120-150 g b.wt) were used in this study and were randomly divided into 6 groups of 7 rats per group, with each animal/group kept in stainless steel cages that were thermostatically-controlled (21 C temperature, 60% humidity) and subjected to a 12-hour light–dark cycle. Animals were randomly assigned a number between 1–6 according to a random number generator in order to assign them to a particular group. Standard food and water was available at all times. The Animal Research Local Ethics Committee of the University of Baghdad/College of Pharmacy approved this study on 10-1-2023 under protocol number 2006.

2.2. Chemicals

Fluorochem (England) supplied the 5-FU and myricetin that were used in the study. a DNA-free total RNA extraction kit called Easy-spinTM was bought from Intron in Korea, for measurement caspas-3, NF-kB P65 and HNF4A by western blot analysis.

2.3. Experimental Groups

Six (6) groups of male rats (N = 7) were included in this study as follows:
Group 1 (control): Rats were orally administered 0.4 milliliters (0.4 ml) of normal saline (NS) containing 5% tween 20 by oral gavage daily for 20 days.
Group 2 (5-FU, 5-fluorouracil)/ (Induction Group): Rats IP-injected with a single dose of 5-FU (150 mg/kg) (Arafah et al. 2022) on the 20th day.
Group 3 (Myricetin, 25 mg/kg/day only): Rats orally administered a myricetin suspension in 0.9% saline at a dosage of 25 mg/kg/day for 20 days by oral gavage.
Group 4 (Myricetin 50 mg/kg/day): Rats orally administered a myricetin suspension in 0.9% saline at a dosage of 50 mg/kg/day for 20 days by oral gavage.
Group 5 (5-FU + Myricetin 25 mg/kg/day): Rats were orally administered a myricetin suspension in 0.9% saline at a dosage of 25 mg/kg/day by oral gavage and a single IP-injected dose of 5-FU (150 mg/kg) was injected on day 20.

Group 6 (5-FU+ Myricetin 50 mg/kg/day): Rats were orally administered a myricetin suspension in 0.9% saline at a dosage of 50 mg/kg/day by oral gavage and a single IP-injected dose of 5-FU (150 mg/kg) was injected on day 20.
One day after the completion of the experiment, the animals in each group were euthanized by diethyl ether and then sacrificed by cervical dislocation.

2.4. Western blot analysis

Reagents and equipments used in Western Blot analysis is shown in **Table 1**.

Table 1. List of reagents and equipment used in Western Blot analysis

Western Blot	
Western Blot Detection Kit	Catalog No: E-IR-R304A, Elabscience Biotechnology Inc., USA
RIPA Lysis Buffer (with PMSF and Na3VO4)	E-BC-R327, Elabscience Biotechnology Inc., China
Excellent Chemiluminescent Substrate Detection Kit	E-BC-R347
Goat Anti Rabbit IgG(H+L) HRP	E-AB-1003
Goat Anti-Mouse IgG (H+L) HRP	E-AB-1001
HNF-4α Monoclonal Antibody	SC-374229
NFκB Polyclonal Antibody	E-AB-32233
Caspase-3 Polyclonal Antibody	E-AB-66940
Beta Actin Polyclonal Antibody	E-AB-40338
PBS Buffer, pH7.4 (10 ×)	E-BC-R187
5 X SDS Loading Buffer	E-BC-R288
Electrophoresis Buffer (10 x)	E-BC-R331
Transmembrane Buffer (10 x)	E-BC-R333
TBST Buffer (10 ×)	E-BC-R335
PVDF Membrane (0.45μm, 8.5×6cm)	E-BC-R266
Albumin fraction V from bovine serum	Thomas Baker, India
Precision Plus Protein™ Dual Color Standards protein Marker (10-250 kDa)	161-0374, BioRad, UK
Super RX X-ray film	Kodak, Rochester, NY, USA
Developer and Fixer reagents	Turkey
Mini-PROTEAN® Tetra Cell and	Catalog No. 1658025, BioRad, UK

PowerPac™ Basic Power Supply	

A Western blot analysis was performed to determine the levels of Caspase-3, NFkBp65, and HNF4alpha; and the technique used for this investigation was a modified from(Willett KL, Roth AR, overview: Hepatotoxicity assessment for botanical dietary supplement .toxicol sci 2004:79;4-9) ; where, sodium orthovanadate (Na3VO4) and phenylmethanesulfonyl fluoride fluoride (PMSF) were added to RIPA lysis buffer after protein samples were isolated from liver tissue; and the BCA Protein Assay Kit (Elabscience, USA) was utilized to determine the protein concentration. Furthermore, 10 microliters of protein extract were transferred to each lane in the subsequent steps, which included isolating the mixture via SDS-PAGE gel and loading it onto PVDF membranes; and before incorporating antibodies (Abs) that attack caspase, NF-KBp65, HNF4 alpha, and beta-actin, the membranes were first incubated with 5% fat-free milk for an hour at ambient temperature; and this was carried out before the serum was added. The subsequent step was to refrigerate the membranes at a temperature of 4°C throughout the entire night; and the horseradish peroxidase-conjugated secondary antibody dilution of 1:10,000 was added to the membranes shortly after being kept at room temperature for one hour which was done through Elabscience. Later, the transcript expression data and photographic representations were correctly identified by combining ECL detection kits (Elabscience, USA) with the ChemiDoc MP Imaging System (Bio-Rad Laboratories, USA). When evaluating the densitometric examination of spectral magnitudes, the one that powers the Image J (NIH) application for image analysis was utilized; and the examination of each specimen was done by the utilization of beta actin/ the internal control.

2.5 Statistical Analysis

Graphic Pad Prism 10 (San Diego, CA, USA) was used for data analysis. The data were displayed by mean ± standard deviation, and were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison tests, with a P-value of less than 0.05 indicating statistical significance.

3. Results

3.1 Effects of Myricetin and 5-fluorouracil on NFKBp65, Caspase-3 and HNF4α Expression in Western Blot Analysis

Figure 1A showed that there was a highly-significant increase (P<0.001) in level of **NF-kBp65** in **group 2** rats**/induction group,** 150mg/kg **5FU** compared to such level in the control/**group 1** rats; furthermore, in **group 3 and 4** rats

(25mg /kg/day myricetin alone) and (50mg /kg/day myricetin alone) respectively, there were non-significant differences (P>0.05) in **NFKBp65** when each compared to such level in **control**/**group 1** rats.

Figure 1A also showed that, there were highly-significant decrease (P<0.001) in **NF-kBp65** level in **groups 5** and **6** that given (myricetin 25mg/kg/day+5FU) and (myricetin 50mg/kg/day +5FU), respectively, and each compared to such level in the toxic **group 2** that IP injected with a single dose 150mg/kg of 5FU.

Concerning Caspase-3, **Figure 1B** showed that there was a highly-significant increase (P<0.001) in level of Caspase-3 in **group 2/**Induction group, 150mg/kg **5FU** compared to the corresponding tissue level in the control/**group 1** rats.

Moreover, there was a significant decrease (P<0.05) in the level of caspase-3 in the **group 3/** (25mg/kg/day myricetin alone) and **group 4/orally-administered myricetin** 50mg/kg/day for 20 days alone each compared to caspase-3 level in control/**group 1** rats. **Figure 4B.**

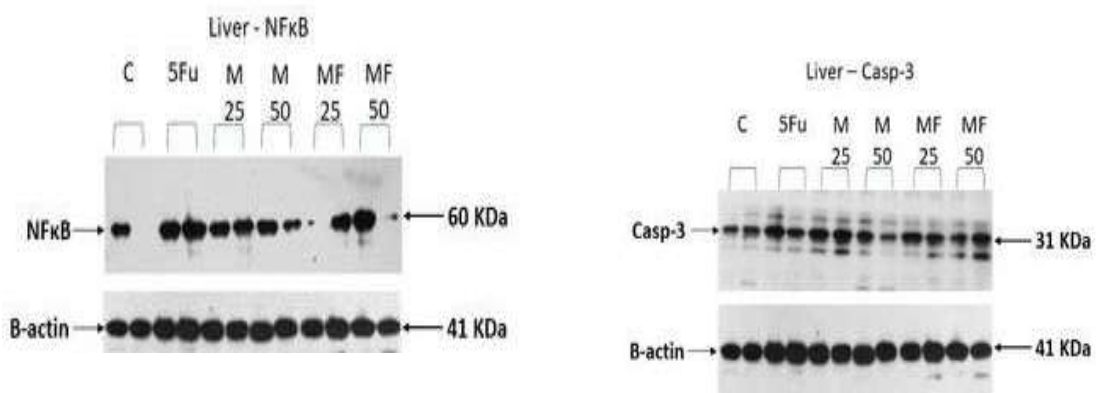
Furthermore, the level of caspase-3 was significantly-decreased (P<0.05) in **group 5 rats /**(myricetin 25mg/kg/day+5FU) and **group 6** rats/(myricetin 50mg/kg/day + 5FU) each compared to the toxic **group 2 rats/Induction group, 150mg/kg** 5FU. **Figure 4B.**

Figure 1C showed that there was a significant decrease (P<0.05) the level of HNF4alpha in **group 2 rats/ (induction group,** 150mg/kg **5-FU)** compared to such level in the control/**group 1** rats.

Furthermore, **Figure 1C** showed there was a significant increase (P<0.05) in the level of HNF4alpha in **group 3/** (25mg/kg/day myricetin alone) and **group 4/** (50mg/kg/day myricetin alone) each compared to the corresponding level in control/**group 1** rats.

Besides, **Figure 1C** showed that there was a significant increase (P<0.05) in level of HNF4alpha in- **group 5/**(myricetin 25mg/kg/day+5FU) and **group 6**/(myricetin 50mg/kg/day +5FU), each compared to such level in the **group 2** rats/IP injected with a single dose 150mg/kg **5-FU.**

A **B**



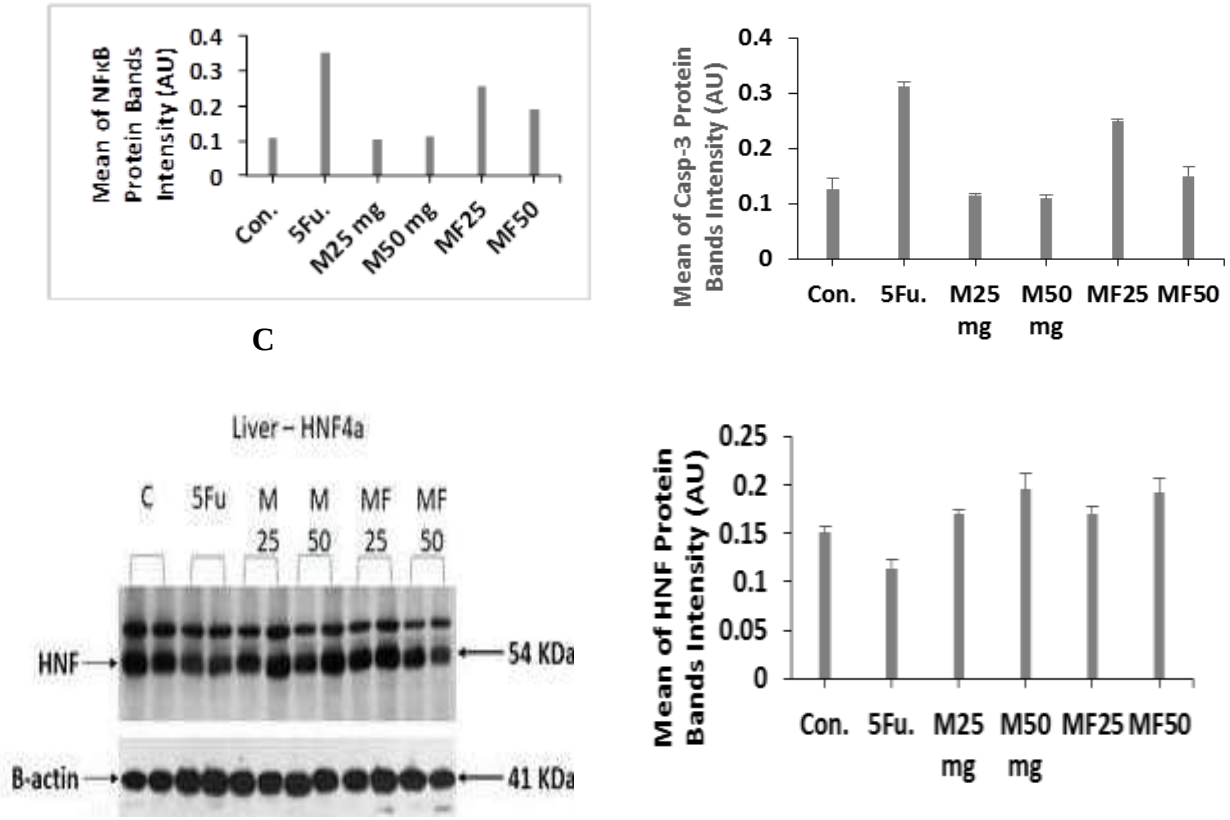


Figure 1. Results of western blot for NFKBp65, caspase-3, and HNF4alpha levels

4. Discussion

Five-fluorouracil (5-FU) is a widely-used chemotherapeutic drug for the treatment of solid tumors (Chalabi-Dchar et al. 2021) but its clinical utility is diminished due to its various toxic effects including hepatotoxicity (Longley et al. 2003). The current study also showed that a single IP dose of **5-FU** (150mg/kg) to male rats **(group 2) Figure 1A**, there was a significant increase ($P<0.05$) in the **NFKBp65** tissue level compared to the corresponding tissue level in controls male (**group 1**) rats.

Furthermore, **Figure 1B** showed that, in male rats IP injected with a single dose of 150mg/kg **5-FU** (**group 2**), such toxic dose of a drug produced a significant ($P<0.05$) increase in **caspase-3** level in rats' liver tissue homogenate compared to the corresponding tissue level in controls male (**group 1**) rats.
Concerning HNF4-alpha, **Figure 1C** showed that there was a significant decrease ($P<0.05$) the level of HNF4alpha in **group 2 rats/ (induction group,** 150mg/kg **5-FU)** compared to such level in the control/**group 1** rats.

Hepatocyte nuclear factors (HNFs) are a group of transcription factors that are primarily expressed in the liver; and are crucial in maintaining metabolic homeostasis by controlling the expression of genes involved in glucose, cholesterol, and fatty acid metabolism (Teschke R. Idiosyncratic DILI: Analysis of 46,266 Cases Assessed for Causality by RUCAM and Published From 2014 to Early 2019. Front Pharmacol. 2019; 10: 730).

In this study, **Figure 1C** showed that the effect of myricetin on the level of HNF4alpha is significantly-increased in **groups 5** and **6** that given (25mg/kg/day+5-FU) and (50mg/kg/day+5-FU), respectively each compared to the corresponding level in **group 2** rats/ 150mg/kg **5 FU** IP injected. This suggests that, myricetin has anti-inflammatory effect against the tissue damage caused by 5-FU; and this is consistent with other research demonstrating myricetin's anti-inflammatory and antioxidant capabilities, which are associated with hepatoprotection against other hepatotoxic compounds (Real M, Barnhill MS, Higley C, Rosenberg J, Lewis JH. DrugInduced Liver Injury: Highlights of the Recent Literature. Drug Saf. 2019; 42(3): 365-387).

5. Conclusion

In conclusion, the current study showed that myricetin protects rats against 5-FU-induced hepatotoxicity in rats; and from the biochemical, molecular, and histological standpoint, the data of this study show that myricetin at the both dose (25mg/kg/day) and (50mg/kg/day) able to reduce 5-FU-induced liver damage, protective effect of myricetin to liver cells through its reduction of inflammatory mediators, apoptotic factors, and OS. Therefore, myricetin may be helpful as a preventive agent to handle hepatotoxicity in liver caused by anticancer medications used. Moreover, there was no previous study concerning the effects of myricetin against 5-FU-induced hepatotoxicity in rats; thus, this study is the first concerning this respect.

6. References

1. Adhab M, et al. (2018) The P1 gene of Cauliflower mosaic virus is responsible for breaking resistance in Arabidopsis thaliana ecotype Enkheim (En-2). Virology 523:15-21
2. Adhab M, et al. (2019) Tracing the lineage of two traits associated with the coat protein of the Tombusviridae: silencing suppression and HR elicitation in Nicotiana species. Viruses 11(7):588

3. Adhab M, Zhang Y, Schoelz J (2023) Transient expression of cauliflower mosaic virus (CaMV) P6-GFP complements a defective CaMV replicon to facilitate viral gene expression, replication and virion formation. Virology 587:109854
4. Agraharam G, Girigoswami A, Girigoswami K (2022) Myricetin: a multifunctional flavonol in biomedicine. Curr Pharmacol Rep 1-14
5. Al-hoshary D, Zalzala MH (2023) Assessment of ellagic acid action in 5-fluorouracil induced intestinal mucositis. Iraqi J Pharm Sci 32(Suppl):33 41. https://doi.org/10.31351/vol32issSuppl.pp33-41
6. Alter P, et al. (2006) Cardiotoxicity of 5-fluorouracil. Cardiovasc Hematol Agents Med Chem 4(1):1-5
7. Arafah A, et al. (2022) Myricetin (3, 3′, 4′, 5, 5′, 7-Hexahydroxyflavone) Prevents 5-Fluorouracil-Induced Cardiotoxicity. ACS Omega 7(5):4514-4524
8. Bharrhan S, Koul A, Chopra K, Rishi P (2011) Catechin suppresses an array of signaling molecules and modulates alcohol-induced endotoxin-mediated liver injury in a rat model. PLoS One 6:e20635
9. Bhattacharya S (2022) Development of 5-FU loaded poly lactic-co-glycolic acid nanoparticles for treatment of lung cancer. Iraqi J Pharm Sci 31(1):130-143
10. Borini P, Guimarães RC (1999) Indicators of inflammation and cellular damage in chronic asymptomatic or oligosymptomatic alcoholics: correlation with alteration of bilirubin and hepatic and pancreatic enzymes. Rev Hosp Clin Fac Med Sao Paulo 54:53-60
11. Burak M, Imen Y (1999) Flavonoids and their antioxidant properties. Turkiye Klinikleri Tip Bilimleri Dergisi 19(1):296-304
12. Chalabi-Dchar M, et al. (2021) A novel view on an old drug, 5-fluorouracil: an unexpected RNA modifier with intriguing impact on cancer cell fate. NAR Cancer 3(3):zcab032. https://doi.org/10.1093/narcan/zcab032
13. Chibber S, et al. White light-mediated Cu (II)–5FU interaction augments the chemotherapeutic potential of 5-FU: an in vitro study. Tumor Biol. 2011;32:881-892. doi:10.1007/s13277-011-0175-4.
14. Choi KC, et al. (2017) Antioxidant, anti-inflammatory and anti-septic potential of phenolic acids and flavonoid fractions isolated from Lolium multiflorum. Pharm Biol 55(1):611-619
15. Chua LS, et al. (2011) Flavonoids and phenolic acids from Labisia pumila (Kacip Fatimah). Food Chem 127(3):1186-1192
16. Conte A, et al. (2013) Is increased blinking a form of blepharospasm? Neurology 80(24):2236-2241

17. Dakhel ZA, Mohammed H (2011) Synthesis of 5-Fluorouracil Derivatives as Possible Mutual Prodrugs with Meloxicam and Ibuprofen for Targeting Cancer Tissues. Iraqi J Pharm Sci 20(2):9-18. https://doi.org/10.31351/vol20iss2pp9-18
18. Fideles LdS, et al. (2020) Role of rutin in 5-fluorouracil-induced intestinal mucositis: prevention of histological damage and reduction of inflammation and oxidative stress. Molecules 25(12):2786
19. Jiang M, et al. (2019) Anti-tumor effects and associated molecular mechanisms of myricetin. Biomed Pharmacother 120:109506
20. Jucá MM, et al. (2020) Flavonoids: biological activities and therapeutic potential. Nat Prod Res 34(5):692-705
21. Kimura Y, Okuda H (1999) Prevention by chitosan of myelotoxicity, gastrointestinal toxicity and immunocompetent organic toxicity induced by 5-fluorouracil without loss of antitumor activity in mice. Jpn J Cancer Res 90(7):765-774
22. Lin J, et al. (2006) Flavonoid intake and colorectal cancer risk in men and women. Am J Epidemiol 164(7):644-651
23. Liu T, et al. (2021) Design, synthesis, antibacterial activity, antiviral activity, and mechanism of myricetin derivatives containing a quinazolinone moiety. ACS Omega 6(45):30826-30833
24. Longley DB, Harkin DP, Johnston PG (2003) 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 3(5):330-338. https://doi.org/10.1038/nrc1074
25. Navarro-Hortal MD, et al. (2020) Role of flavonoids against adriamycin toxicity. Food Chem Toxicol 146:111820
26. Nija B (2023) Prodrug Approach: NSAID Conjugates of Platinum Compounds as Anti-Cancer Agents. Iraqi J Pharm Sci 32(3):1-13
27. Ong KC, Khoo HE (1997) Biological effects of myricetin. Gen Pharmacol 29(2):121-126
28. Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47
29. Pervin M, Karim MR, Kuramochi M, Izawa T, Kuwamura M, Yamate J (2018) Macrophage populations and expression of regulatory inflammatory factors in hepatic macrophage-depleted rat livers under lipopolysaccharide (LPS) treatment. Toxicol Pathol 46:540-552
30. Ramesh P, et al. (2021) Flavonoids: classification, function, and molecular mechanisms involved in bone remodeling. Front Endocrinol (Lausanne) 12:779638

31. Real M, Barnhill MS, Higley C, Rosenberg J, Lewis JH. DrugInduced Liver Injury: Highlights of the Recent Literature. Drug Saf. 2019; 42(3): 365-387.
32. Ross JA, Kasum CM (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 22(1):19-34
33. Salimi A, Jamali Z, Shabani M (2021) Antioxidant potential and inhibition of mitochondrial permeability transition pore by myricetin reduces aluminium phosphide-induced cytotoxicity and mitochondrial impairments. Front Pharmacol 12:719081
34. Schwab M, et al. (2008) Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol 26(13):2131-2138. https://doi.org/10.1200/JCO.2007.15.0177
35. Shihab IK, Mohammed MH (2019) Synthesis, Characterization and Preliminary Anticancer Study of Novel 5-fluorouracil Conjugate with Pyrrolidine Dithiocarbamate as a Mutual Anticancer Prodrug. Iraqi J Pharm Sci 28(2):17-23
36. Sklenarova R, et al. (2021) Effect of the natural flavonoids myricetin and dihydromyricetin on the wound healing process in vitro. J Appl Biomed 19:149-158
37. Spolarics Z (1998) Endotoxemia, pentose cycle, and the oxidant/antioxidant balance in the hepatic sinusoid. J Leukoc Biol 63:534-541
38. Tanaka F, Fukuse T, Wada H, Fukushima M (2000) The history, mechanism and clinical use of oral 5-fluorouracil derivative chemotherapeutic agents. Curr Pharm Biotechnol 1(2):137-164. https://doi.org/10.2174/1389201003378979
39. Tveden-Nyborg P, Bergmann TK, Jessen N, Simonsen U, Lykkesfeldt J (2023) BCPT 2023 policy for experimental and clinical studies. Basic Clin Pharmacol Toxicol 133(4):391-396. https://doi.org/10.1111/bcpt.13944
40. Teschke R. Idiosyncratic DILI: Analysis of 46,266 Cases Assessed for Causality by RUCAM and Published From 2014 to Early 2019. Front Pharmacol. 2019; 10: 730.
41. Yao Y, et al. (2014) Preformulation studies of myricetin: a natural antioxidant flavonoid. Pharmazie 69(1):19-26
42. Yousef HN, Aboelwafa HR (2017) The potential protective role of taurine against 5-fluorouracil-induced nephrotoxicity in adult male rats. Exp Toxicol Pathol 69(5):265-274
43. Zhang N, et al. (2018) Myricetin attenuated LPS induced cardiac injury in vivo and in vitro. Phytother Res 32(3):459-470

44. Willett KL, Roth AR, overview: Hepatotoxicity assessment for botanical dietary supplement .toxicol sci 2004:79;4-9