

## Evaluation of The Effect of Fisetin against Cyclophosphamide-Induced Myelosuppression and Oxidative Stress in Male Albino Rats

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### Abstract

Myelosuppression is a serious disease that is related to the malfunction of blood cells production that leads to cytopenia which is the most serious hematologic toxicity of cancer chemotherapies including cyclophosphamide, which is a strong oxazaphosphorine [a nitrogen mustard alkylating agent] that can be used alone or combined with other chemotherapeutic agents for the treatment of different malignant diseases. It induces severe bone marrow suppression by damaging hematopoietic stem cells through the generation of oxidative stress. Fisetin is a hydrophobic polyphenolic compound with a wide range of pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, osteoprotective, antidiabetic, and anti-carcinogenic activities. The present study aims to evaluate the effects of fisetin alone and pretreatment with fisetin followed by cyclophosphamide on some selected hematological and oxidative stress parameters in the male rats' model. Animals were randomly divided into 4 groups each with 7 rats. **The first group** received only the vehicle 1% dimethyl sulfoxide (negative control group). **The second group** received oral fisetin. **The third group** received a single intraperitoneal (IP) injection of cyclophosphamide (CP) and the **fourth group** received fisetin for 7 constitutive days then a single IP injection of CP on day 7. Results showed that both fisetin and CP significantly reduced total white blood cells and platelet counts compared to such counts in negative control/**Group I** ( $P<0.05$ ) when each was administered alone and in combination. Furthermore, results viewed that fisetin significantly increased GSH and SOD1, and decreased MDA levels in serum compared to such levels in CP-injected rats (**Group III**) ( $P<0.05$ ). The study concluded that the administration of fisetin alone causes leukopenia and thrombocytopenia and this decrease was augmented in combination with CP; while exhibiting a strong antioxidant effect against CP-induced oxidative stress.

**Keywords:** Fisetin, Cyclophosphamide, Myelosuppression, Oxidative Stress

### تقييم تأثير الفيزيتين ضد تثبيط نخاع العظم والإجهاد التأكسدي الناجم عن السيكلوفوسفاميد في ذكور الجرذان البيضاء

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### الخلاصة

ان كبت نخاع العظم هو مرض خطير مرتبط بخلل في إنتاج خلايا الدم التي تؤدي إلى قلة كريات الدم البيضاء التي تعد سمية دموية خطيرة التي تسببها العلاجات الكيميائية للسرطان بما في ذلك السيكلوفوسفاميد وهو أوكساسافوسفورين [عامل مؤكل بخردل النيتروجين] الذي يستخدم بمفرده أو مع عوامل العلاج الكيميائي الأخرى لعلاج مجموعة متنوعة من الأمراض السرطانية حيث يؤدي إلى تثبيط شديد لنخاع العظم عن طريق إتلاف الخلايا الجذعية المكونة للدم من خلال توليد الإجهاد التأكسدي. الفيزيتين هو مركب بوليفينولي مقاوم للماء، وله مجموعة واسعة من الخصائص الدوائية مثل مضادات الأكسدة، ومضادات الالتهابات، ومضادات الميكروبات، والعظام، ومضادات السكر، ومضاد للسرطان. تهدف هذه الدراسة إلى تقييم تأثيرات الفيزيتين بمفرده وقبل السيكلوفوسفاميد على بعض متغيرات الدم والأكسدة المختارة في نموذج ذكور الجرذان. تم تقسيم الحيوانات بشكل عشوائي إلى 4 مجموعات في كل منها 7 جرذان. تلقت المجموعة الأولى 1٪ ثنائي ميثيل سلفوكسيد (مجموعة السيطرة السلبية). تلقت المجموعة الثانية فيزييتين بمفرده وقبل السيكلوفوسفاميد وتلقت المجموعة الرابعة فيزييتين عن طريق الفم لمدة 7 أيام متتالية والسيكلوفوسفاميد الذي حقن داخل الصفاق في اليوم السابع. أظهرت النتائج أن كلا من الفيزيتين والسيكلوفوسفاميد قلل بصورة معنوية من إجمالي عدد خلايا الدم البيضاء والصفائح الدموية مقارنة بتلك الأعداد في مجموعة السيطرة السلبية / المجموعة الأولى. علاوة على ذلك، أظهرت النتائج أن الفيزيتين زاد بصوره معنويه من مستويات الكلوتاتيون وانزيم السوبر أوكسيد الغير منتقل 1 مع خفض مستوى المالونديالدهايد في مصل الدم مقارنة بتلك المستويات في جرذان المجموعة الثالثة. يمكن الاستنتاج بأن إعطاء الفيزيتين وحده يسبب قلة الكريات البيض ونقص في عدد الصفائح الدموية وهذا النقص يعزز بواسطة السيكلوفوسفاميد بينما اظهر الفيزيتين تأثيرًا قويًا ضد الإجهاد التأكسدي الناتج عن السيكلوفوسفاميد.

الكلمات المفتاحية: فيزييتين ، سيكلوفوسفاميد ، كبت نخاع العظم ، الإجهاد التأكسدي

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## Introduction

The bone marrow (BM) is considered the primary source for the production of blood and plasma components (hematopoiesis), including myeloid and lymphoid progenitor cells. Moreover, a such process primarily occurs in the axial skeleton<sup>(1)</sup>. Myeloid hematopoietic progenitor cells normally arise from multipotent hematopoietic stem cells (HSCs) that can self-renewal, permitting continuous replacement of granulocytes, macrophages, and erythrocytes<sup>(2)</sup>.

The spleen is a large secondary lymphoid organ found in all vertebrates and primarily functions as a blood filter and it is a sentinel organ for lymphocytes circulation<sup>(3)</sup>. It is composed of two compartments: the red pulp (RP) which contains leukocytes with innate immune functions and the white pulp (WP) which contains lymphocyte cells with adaptive immune functions<sup>(4)</sup>.

Myelosuppression is a serious disease that is related to defects in blood cells production which destroys human body functions, thereby affecting the patient's quality of life<sup>(5)</sup>, and this can result in cytopenia [fewer red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs)], and as a result of an imbalance of production versus destruction or loss of RBCs, the blood has decreased capacity for carrying hemoglobin (Hb) and oxygen<sup>(6)</sup>. It is the most serious hematologic toxicity related to cancer chemotherapy leading to chemotherapy dose limitations that can be tolerated<sup>(7)</sup>.

Cyclophosphamide (CP) is a strong oxazaphosphorine [a nitrogen mustard alkylating agent] used alone or combined with other chemotherapeutic agents for the treatment of different malignant diseases. Furthermore, it is an immunosuppressant agent that is increasingly used for the treatment of certain autoimmune diseases either as a sole agent or in combination with corticosteroids<sup>(8)</sup>. The metabolite of the such chemotherapeutic agent is acrolein, which can induce oxidative stress (OS) and can lead to DNA damage of normal cells in addition to different organ toxicities<sup>(9)</sup>.

The plant-extracted compounds are reported to have the potential in regulating many processes in chronic diseases including inflammation, cancer, and metabolic and degenerative diseases<sup>(10)</sup>. Fisetin (3,3',4',7-tetrahydroxyflavone) is a hydrophobic polyphenolic compound found primarily in strawberries, and other fruits and vegetables including apples, spinach, blueberry, grape, persimmon, kiwi, onion, and cucumber<sup>(11)</sup>.

Several studies reported that fisetin has a broad range of pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, osteoprotective, anti-diabetic, and anti-carcinogenic activities<sup>(12)</sup>.

Due to its multiple bioactive properties, fisetin is now considered a health-promoting factor<sup>(13)</sup>. The structural features of fisetin as well as its ability to modulate certain cellular signaling pathways may be related to its antioxidant activity<sup>(14)</sup>.

The present study was designed to evaluate the effect of fisetin flavonoid orally-administered against CP-induced myelosuppression and OS in male albino rats.

## Materials and Methods

### Chemicals and their suppliers

Cyclophosphamide (CP) vial 500mg purchased from Baxter, Germany; fisetin pure powder was purchased from Hangzhou Hyper Chemicals Limited/China; dimethyl sulfoxide (DMSO) and diethyl ether was purchased from Alpha chemika company/ India; Formalin 37% was purchased from Sinopharm chemical reagent Co., Ltd, China; Rats Malondialdehyde ELISA kit, reduced glutathione (GSH) ELISA kit and rat Superoxide dismutase 1 (Cu-Zn) ELISA kit, were purchased from Bioassay Technology Laboratories, China.

### Preparation of fisetin stock solution

Fisetin solution was prepared by dissolving 30mg of the pure powder of fisetin in 0.1ml DMSO and then diluted with glycerin oil up to 10ml to get a concentration of 1% DMSO in glycerin. The dose of fisetin used is 10 mg/kg/day and was orally administered via rats' oral gavage<sup>(15)</sup>.

### Experimental protocol

This study was approved by the Ethical Committee of the Department of Pharmacology and Toxicology and the Scientific Committee of the College of the Pharmacy/University of Baghdad. Twenty-eight (28) healthy male Albino rats weighing 150-220gm were got from Animal House of the College of Pharmacy and were kept in the Animal House under conditions of controlled temperature  $20\pm 5^{\circ}\text{C}$ , and fed commercial pellets and the tap water *ad libitum*. Rats were accommodated for one week before starting the experiment which is last for 7 constitutive days.

The healthy Experimental Albino rats were arbitrarily divided into 4 groups (7 animals/group) as follows:

**Group I:** Rats orally administered diluted 1% DMSO solution (the same vehicle system used to dissolve fisetin powder) via oral gavage for 7 days. This group represents the **negative control** group.

**Group II:** Rats orally-administered fisetin at a dose (10mg/kg/day) dissolved in diluted dimethyl sulfoxide 1% (DMSO) solution alone via oral gavage for 7 consecutive days<sup>(15)</sup>.

**Group III:** Rats IP-injected with a single dose of cyclophosphamide (150mg/kg)<sup>(16)</sup>

**Group IV:** Rats orally-administered fisetin by oral gavage in the early morning at 9.00 a.m. daily at a

dose (10mg/kg/day) dissolved in 1% (DMSO) for 7 consecutive days and a single IP injection of (150mg/kg) of cyclophosphamide, which is administered at day 7.

Each rat was euthanized by using diethyl ether on day 8 at 9 am and animals were killed by decapitation.

#### Estimation of blood hematology parameters

Blood was withdrawn from the rat's external jugular vein of the neck and put in the ethylenediaminetetraacetic acid (EDTA)-coated tube for estimation of total-red blood cells (T-RBCs), total-white blood cells (T-WBCs), platelets (PLTs) count and hemoglobin (Hb) concentration on the same day by utilizing the automated hematology analyzer (Mindray BC-5000) <sup>(17)</sup>.

#### Quantitative analysis of serum oxidative stress (os) parameters in rats

Blood was put in a clot activated-gel tube and centrifuged at 3000 rpm for 20 minutes; then the serum was collected for quantitative measurement of OS parameters Malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase-1 (SOD1) enzyme activity following the principle of sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method <sup>(18)</sup>.

#### Histological examination of rat's spleen

A small upper part of the rat's spleen was carefully removed and washed with normal saline (N/S); then, tissue fixation was done in 10% formaldehyde solution. Sections of spleen tissue were prepared according to the method of Junqueira LC. *et al* <sup>(19)</sup> using the paraffin sections method and were stained with hematoxylin and eosin (H&E) and examined using a light microscope.

#### Statistical analysis

Data were analyzed by utilizing the computerized statistical package for the social sciences (SPSS) version 26. Means of groups were compared using one-way analysis of variance (ANOVA) followed by Post hoc (Tukey) for normally distributed values and the data expressed as mean  $\pm$  standard deviation (SD) and were considered significant at a *P*-value less than 0.05 ( $p < 0.05$ ). For not normally distributed data, medians of groups were compared using non-parametric Kruskal-Wallis and Mann-Whitney tests, and data expressed as median  $\pm$  interquartile range and were considered significant at *P*-value less than 0.05 ( $p < 0.05$ ).

## Results

Table 1 showed that male rats with orally-administered fisetin (**Group II**) exhibited a significant decrease ( $P < 0.05$ ) in T-WBCs and PLTs counts and a significant increase ( $p < 0.05$ ) in Hb levels and non-significant differences in Total-

RBCs counts as compared with such counts in negative control rats (**Group I**) ( $P > 0.05$ ). Also, Table 1 showed that in rats IP injected with CP (**Group III**) there was a significant reduction ( $P < 0.05$ ) in T-WBCs, and PLTs counts while exhibiting a significant increase in Hb levels, but there was a non-significant difference ( $P > 0.05$ ) in T-RBCs counts in comparison to aforementioned hematology parameters in rats of negative control (**Group I**).

Furthermore, Table 1 showed that there were non-significant differences ( $P > 0.05$ ) in the T-WBCs and T-RBCs counts in male rats of **Group IV** (rats orally-administered fisetin before CP); while exhibited a significant decrease ( $P < 0.05$ ) in the PLTs count and Hb levels compared to that in **Group III** rats.

In Table 2, results showed that there was a non-significant difference ( $P > 0.05$ ) in serum GSH level in male rats with orally-administered fisetin (**Group II**) compared to the corresponding serum level in negative control rats (**Group I**); while the IP injection of CP (**Group III**) caused significant decrease ( $P < 0.05$ ) in such serum level compared to corresponding level in control (**Group I**) rats. Furthermore, Table 2 showed that pretreatment of rats with fisetin before CP (**Group IV**) caused a significant increase ( $P < 0.05$ ) in serum GSH level compared to the aforementioned serum level in rats IP injected with a single dose of CP (**Group III**).

Moreover; Table 2 showed that there was a non-significant difference ( $P > 0.05$ ) in the serum MDA level in rats of **Group II**/orally-administered fisetin compared to such serum level in control (**Group I**) rats; while IP injection of male rats with CP (**Group III**) caused a significant ( $P < 0.05$ ) increase in the serum MDA level compared to the corresponding serum level in control rats/ (**Group I**).

Furthermore, Table 2 showed that in **Group IV** (rats of fisetin before CP), there was a significant ( $P < 0.05$ ) decrease in the serum MDA level compared to such level in rats of **Group III**/IP injected with a single dose of CP.

Meanwhile, data presented in Table 2 showed that in (**Group II**) rats there was a non-significant ( $P > 0.05$ ) difference in serum SOD1 enzyme level compared to such enzyme level in serum of control rats/**Group I**; while IP injection with CP (**in Group III rats**) caused a significant decrease ( $P < 0.05$ ) in serum SOD1 enzyme level compared to the corresponding serum enzyme level in control rats/**Group I**.

Additionally, Table 2 showed that in **Group IV**, there was a significant ( $P < 0.05$ ) increase in serum SOD1 enzyme level (3.84 ng/ml) compared to the mean serum enzyme level (3.33 ng/ml) in rats IP injected with a single dose of CP (**Group III**).

**Table 1. The effect of fisetin alone and pretreatment with cyclophosphamide on male albino rats' hematology parameters.**

Animal Groups	Total WBCs count (cell/mm <sup>3</sup> ) median $\pm$ interquartile range	RBCs count (Cell/mm <sup>3</sup> ) median $\pm$ interquartile range	Platelets count(cell/mm <sup>3</sup> ) (Mean $\pm$ SD)	Hemoglobin level (g/dl) (Mean $\pm$ SD)
Negative Control (Group I)	8900 $\pm$ 2600 <sup>a</sup>	5770000 $\pm$ 1750000 <sup>a</sup>	866428.57 $\pm$ 167813.24 <sup>a</sup>	10.6 $\pm$ 1.60 <sup>a</sup>
Fisetin 10mg/kg (Group II)	4800 $\pm$ 3500 <sup>b</sup>	7000000 $\pm$ 4660000 <sup>a</sup>	388571.42 $\pm$ 155903.66 <sup>b</sup>	12.2 $\pm$ 0.98 <sup>b</sup>
Cyclophosphamide 150mg/kg (Group III)	1800 $\pm$ 1000 <sup>c</sup>	8070000 $\pm$ 1660000 <sup>a</sup>	628857.14 $\pm$ 83636.97 <sup>c</sup>	14.4 $\pm$ 1.07 <sup>c</sup>
Fisetin and cyclophosphamide (Group IV)	2500 $\pm$ 300 <sup>c</sup>	6890000 $\pm$ 1160000 <sup>a</sup>	137428.57 $\pm$ 145536.77 <sup>d</sup>	12.7 $\pm$ 1.68 <sup>d</sup>

Each value is represented by median  $\pm$  interquartile range by Kruskal-Wallis and Mann whiney test and mean $\pm$  SD by one-way ANOVA tests.

-Values with non-identical small letters (a, b, c, and d) are significantly different ( $P < 0.05$ ).

-Values with an identical small letter (a) are non-significantly-different ( $P > 0.05$ )

**Table 2. The effect of fisetin alone and pretreatment with cyclophosphamide on selected serum OS parameters.**

Animal groups	Serum GSH level (mg/L)	Serum MDA level (mmole/ml)	Serum SOD level (ng/ml)
Negative Control (Group I)	201.06 $\pm$ 13.63 <sup>a</sup>	1.09 $\pm$ 0.20 <sup>a</sup>	4.38 $\pm$ 0.39 <sup>a</sup>
Fisetin 10 mg/kg (Group II)	191.13 $\pm$ 17.09 <sup>a</sup>	1.04 $\pm$ 0.13 <sup>a</sup>	3.92 $\pm$ 0.34 <sup>a</sup>
Cyclophosphamide 150 mg/kg (Group III)	174.88 $\pm$ 11.70 <sup>b</sup>	1.32 $\pm$ 0.13 <sup>b</sup>	3.33 $\pm$ 0.47 <sup>b</sup>
Fisetin and cyclophosphamide (Group IV)	219.44 $\pm$ 27.70 <sup>a</sup>	1.09 $\pm$ 0.22 <sup>a</sup>	3.84 $\pm$ 0.43 <sup>a</sup>

Data were expressed as Mean  $\pm$ SD by a one-way ANOVA test.

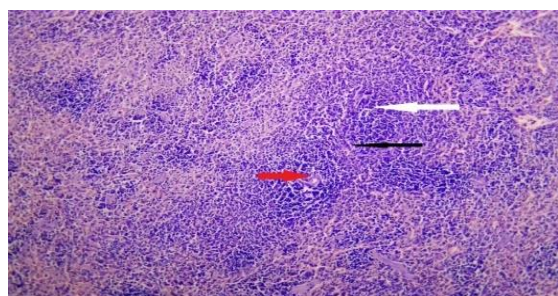
- Values with non-identical small letters (a, and b) are significantly different ( $P < 0.05$ ).

-Values with an identical small letter (a) are non-significantly different ( $P > 0.05$ ).

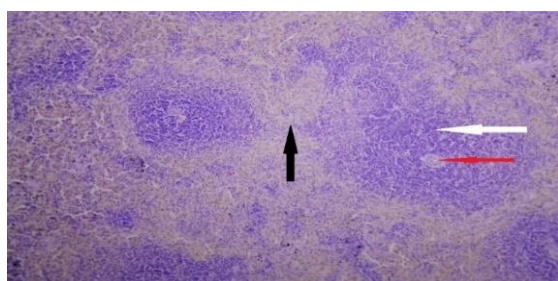
#### **Histological examination of the rats' spleen**

Figure -1A showed the spleen structural area of the control **Group I** rats that are (characterized by the normal appearance of the white pulp (WP), the red pulp (RP), and the central arteriole). Figure-1B belongs to **Group II** rats (orally-administered fisetin at dose 10mg/kg/day); where there was a reduction in the WP with a widening of RP. Besides, in the section of male rats' spleen, IP injected with CP (**Group III**) (Figure -1C), there was a reduction in

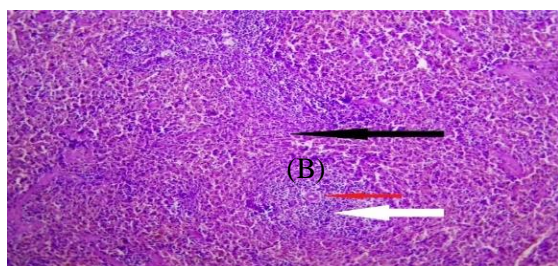
the WP that was represented by the destruction of lymphocytes. Additionally, in the spleen section of experimental **Group IV** rats (orally-administered fisetin daily at a dose of 10mg/kg/day before a single IP injection of CP), there is more reduction in the WP by decreasing in the intensity of lymphocytes with a widening of RP as shown in (Figure-1D).



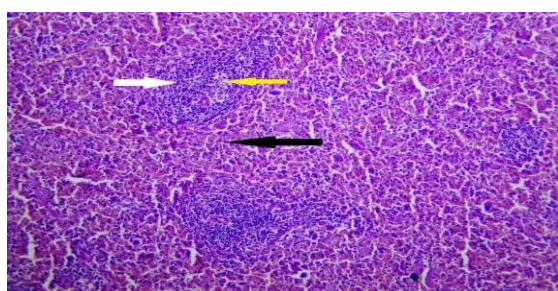
(A)



(B)



(C)



(D)

**Figure 1. Histological examination of male rats' spleen ;(A) Control group; (B) Fisetin 10 mg group (C) Cyclophosphamide group;(D) Fisetin and cyclophosphamide group;(; White arrow refers to the white pulp; Black arrow refers to the red pulp. The yellow arrow refers to destructive lymphocytes; the red arrow refers to the central arteriole.**

## Discussion

Cyclophosphamide (CP) is a prodrug that's converted to two active metabolites, phosphoramidate mustard (PM) (an anticancer metabolite) and a toxic metabolite called acrolein, which is responsible for hematological and myelotoxicity <sup>(20)</sup>.

Results of Table 1 showed that the oral fisetin administration in rats of Group II caused a significant decrease in T-WBCs and PLTs counts compared to such counts in control rats (Group I). These results are consistent with that of others; where fisetin exhibited a significant decrease in T-WBCs counts in the peripheral blood of bleomycin (rather than CP utilized in the current study) which induced idiopathic pulmonary fibrosis in mice <sup>(21)</sup> with the lack of similar researches published previously for rats. Furthermore, the immunosuppression of T-lymphocyte cells by fisetin may be related to the inhibition of activation of the nuclear factor-kappa B (NF- $\kappa$ B) by suppressing their upstream signaling molecules in cancer cells <sup>(22)</sup>.

Additionally, in rats of [Group III/CP], Table 1 showed a significant decrease in T-WBCs and PLTs counts in comparison with that in control rats/ Group I; and these results confirm the myelosuppressive effect of CP which can be related to the imbalance in the OS process that was consistent with other researchers <sup>(23)</sup>.

On the other hand, in the present study, the oral administration of fisetin before CP (Group IV) to rats caused a significant decrease in PLTs count compared with the aforementioned count in (Group III rats/CP), and the suggestion is that fisetin has an inhibitory effect on the corresponding blood cells in addition to the inhibition promoted by CP.

As mentioned previously, CP and its metabolites can react with reduced glutathione (GSH) and restrict the antioxidant activity; and it also causes lipid peroxidation by increasing the production of ROSs leading to OS <sup>(24)</sup>.

Oxidative stress (OS) is defined as an imbalance between the production and scavenging of ROSs in the body leading to disrupts its ability to detoxify reactive intermediates or to repair the organs and cellular systems that can be damaged by the ROS <sup>(25)</sup>. It appears that the use of antioxidant compounds should be capable of inhibiting the harmful effects of ROS resulting from using chemotherapy drugs <sup>(26)</sup>.

The results of the present study (Table 2) showed that CP injection intraperitoneally injection (Group III rats) caused a significant decrease in serum level of reduced GSH and SOD 1 levels while significantly increasing the MDA level in comparison with such levels in control rats/ Group I; this can confirm the role of CP in disturbing the cellular redox state by generating OS conditions <sup>(27,28)</sup>

Also, the results of Table 2 showed that in rats of Group IV with orally-administered fisetin before CP injection there was a significant increase in serum GSH and SOD 1 levels and a significant decrease in MDA level as compared with the corresponding levels in CP injected rats (Group III) and this may confirm the antioxidant potential of fisetin against

CP-induced OS and agreed with what previously mentioned about the antioxidant activity of fisetin against cigarette smoked -induced OS in rats <sup>(29)</sup>.

Several studies have reported that fisetin has antioxidant activity and other pharmacological properties <sup>(13)</sup>. Furthermore, researchers stated that the most antioxidant activity of fisetin contributes to its 3-OH group in addition to the 3'-OH,4'-OH groups and the double bond between carbon 2 - and 3 which are capable of enhancing antioxidant activity <sup>(30)</sup>.

In addition, Basu A and his colleagues (2016) reported that fisetin from strawberries elevated the plasma antioxidant markers including [catalase (CAT), glutathione peroxidase (GPx), and reductase enzymes] and whole blood [GSH] in obese adults with the elevation of serum lipids <sup>(31)</sup>.

The present study examined the effect of fisetin on rats' spleen histological features (Figure 1); where the oral administration of fisetin alone (**Group II**) (Figure 1B) exhibited a reduction in the WP as compared with the negative control/**Group I** (Figure 1A). This may result from the inhibitory effect on T lymphocyte cells by fisetin via inhibition of the activation of NF-kB through suppressing their upstream signaling molecules as mentioned in a previous study in cancer cells <sup>(22)</sup>. On the other hand, researchers found that fisetin promoted cell death through mitochondrial apoptosis, arrest of the cell cycle, and inhibition of cell migration or via the mediation of multiple signaling pathways <sup>(32,33)</sup>. Moreover, other researchers showed that fisetin had immunosuppressive function through its effect on T-lymphocyte cells leading to reduce the total number of lymphocytes in samples of spleen tissue <sup>(34)</sup>.

Additionally, in the splenic histology of **Group III** rats with IP injection of a single dose of CP (Figure 1C), there was a reduction in the WP which may be related to OS generated by CP and its metabolites, which can damage the splenic cells as previously mentioned by others <sup>(23)</sup>.

Furthermore, the splenic histology of rats with orally-administered fisetin before CP (**Group IV**) (Figure 1D), showed that there was an additional reduction in both WP and RP diameters. The suggestion for such effects may be related to the inhibitory effects of both compounds on splenic cells by different mechanisms.

## Conclusion

According to the results obtained from the present study, it can be concluded that the administration of fisetin alone causes leukopenia and thrombocytopenia, which are more intense in combination with CP; while exhibiting a strong antioxidant effect against CP induced-OS.

## Limitations

In the current study, female rats were not included in the present study; since results were restricted to male rats only. Second: measurements

of OS parameters in tissue homogenates were not investigated which could have additional perceptions about the antioxidant potential of fisetin.

## Recommendations for future works

The authors recommend the following notes for future works. **First:** *In vivo* studies using large-scale animals of both sexes to assess the role of using fisetin alone in different organs in normal and in pathological cases and with other drugs. **Second:** Evaluate the antioxidant potential of fisetin in different tissue homogenates; and **Third:** Investigate other roles of fisetin as possible -anti-apoptotic and -anti-inflammatory effects against CP-induced myelosuppression.

## Funding

The authors declare that there were no financial supports for this research.

## Conflicts of Interest

The authors declare that there is no conflict of interest

## Ethics Statements

This study was approved by the Ethical and the Scientific Committee of the Department of Pharmacology and Toxicology of the College of the Pharmacy/University of Baghdad at January 2022.

## Author Contribution

The first author contributed to the experimental work, data gathering, analysis and interpretation of the results and writing of the manuscript under the supervision of the second author who presented the research idea, developed the design and reviewed the article.

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