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Evaluating the Effects of Different Doses of Vitamin B2 and Single Dose of Vitamin B12 Against Myelosuppression Induced by Cyclophosphamide in Experimental Rats

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Abstract

Cyclophosphamide is chemotherapeutic agent that is utilized for the treatment of different malignancies; however its'use can be associated with numerous adverse effects. Vitamin B2 and vitamin B12 suggested having myeloprotective effect. This work is designed to investigate the myeloprotective effect of both vitamins against myelosuppression induced by cyclophosphamide. One hundred adult rats of both sexes were used in this study. The animals were randomly enrolled into ten groups of 10 rats per each. Group I: Control group. Group II: Cyclophosphamide-treated. Group III and Group IV Orally-administered of vitamin B2 (10, and 40 mg/kg/day), respectively alone for 7 days. Group V: Orally-administered vitamin B12 (0.1 mg/kg/day) alone for 7 days. Group VI and Group VII: Orally-administered vitamin B2 (10, and 40 mg/kg/day), respectively for 7 days and a single intraperitoneal injection of cyclophosphamide (150 mg/kg) at day 7. Group VIII: Orally-administered vitamin B12 (0.1 mg/kg/day) for 7 days and a single intraperitoneal injection of cyclophosphamide (150 mg/kg) at day 7. Group IX: Orally-administered a combination of vitamin B2 (10 mg/kg/day) and vitamin B12 (0.1 mg/kg/day) for 7 days and a single intraperitoneal injection of cyclophosphamide (150 mg/kg) at day 7. Group **X:** orally-administered a combination of vitamin B2 (40 mg/kg/day) and vitamin B12 (0.1 mg/kg/day) for 7 days and a single intraperitoneal injection of cyclophosphamide (150 mg/kg) at day 7. On day eight, animals were sacrificed and blood collected for complete blood counts and femur bone were extracted for bone marrow histological examination. Vitamin B2 and vitamin B12 significantly (P<0.05) increase complete blood counts; and the combination of vitamins produce -a significant (P<0.05) increase in complete blood counts compared to corresponding counts in other Groups, and -improve histopathological changes compared to Group II rats. In conclusion both vitamins may have myeloprotective effects against cyclophosphamide-induced myelosuppression.

Key words: Cyclophosphamide, Vitamin B2, Vitamin B12, Myelosuppression, Rats.

تقييم تأثير الجرع المختلفة من فيتامين ب٢ والجرعة الواحدة من فيتامين ب ١٢ ضد كبت نخاع العظم الناجم عن عقار سايكلوفوسفامايد في الجرذان المختبرية

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الخلاصة

عقار السايكلوفوسفامايد يستخدم لمعالجة انواع مختلفة من السرطان لكن قد يصاحب استخدامه اضرار جانبية كثيرة. فيتامين ب ٢ وفيتامين ب ١٢قد يمتلكان القدرة على حماية نخاع العظم. لذلك كان الهدف من هذه الدراسة هو تقييم تأثير فيتامين ب٢ و ب١٢ ضد سمية عقار سايكلوفوسفامايد على نخاع العظم. تم استخدام ١٠٠ جرذ بالغ من كلا الجنسين حيث قسمت الى عشر مجاميع وكل مجموعة تحتوي على ١٠ جرذان. المجموعة الاولى: حقنت ب محلول ملحي لمدة ٧ أيام, المجموعة الثانية: حقنت ب ١٥٠ ملغم/كغم سايكلوفوسفامايد, المجموعة الثالثة و الرابعة: اعطيت ١٠و ٤٠ ملغم/كغم فيتامين ب٢ تباعا عن طريق الفم لمدة ٧ ايام, المجموعة الخامسة: اعطيت ٠,١ ملغم/كغم من فيتامين ب ١٢ عن طريق الفم لمدة ٧ ايام, المجموعة السادسة و السابعة: اعطيت ١٠و ٤٠ ملغم/كغم فيتامين ب٢ تباعا عن طريق الفم لمدة ٧ ايام وحقنت ب ١٥٠ ملغم/كغم سايكلوفوسفامايد في اليوم السابع, المجموعة الثامنة: اعطيت ٠,١ ملغم/كغم فيتامين ب١٢ عن طريق الفم لمدة ٧ ايام وحقنت ب ١٥٠ ملغم/كغم سايكلوفوسفامايد في اليوم السابع, المجموعة التاسعة: اعطيت ١٠ ملغم/كغم فيتامين ب٢ و ٠,١ ملغم/كغم فيتامين ب١٢عن طريق الفم لمدة ٧ ايام وحقنت ب ١٥٠ ملغم/كغم سايكلوفوسفامايد في اليوم السابع, المجموعة العاشرة: اعطيت ٤٠ ملغم/كغم فيتامين ب٢ و ٠,١ ملغم/كغم فيتامين ب١٢عن طريق الفم لمدة ٧ ايام وحقنت ب ١٥٠ ملغم/كغم سايكلوفوسفامايد في اليوم السابع. في اليوم الثامين تم التضحية بالجرذان لغرض جمع الدم لمعرفة تعداد كريات الدم وجمع العظم للحصول على نخاع العظم ودراسة التغيرات النسيجية.بينت النتائج ان فيتامين ب٢ و ب١٢ انتجا زيادة ملحوظةفي حساب كريات الدم والمزج بينهما انتج زيادة معنوية في حساب كريات الدم بالمقارنة مع المجموعة الثانية فضلا عن انتاج تحسن ملحوظ لنسيج نخاع العظم. قد يكون لكل من فيتامين ب٢ و ب١٢ القدرة على حماية نخاع العظم ضد سمية عقار سايكلوفوسفامايد على نخاع العظم

الكلمات المفتاحية: سايكلوفوسفامايد, فيتلمين ب٢, فيتامين ب ١٢, تثبيط العظم, الجرذان.

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Introduction

Cyclophosphamide (CPA) among the most widely used chemotherapeutic drug to kill cancer cells [(1)]. Such drug is used alone or in combination with other chemotherapeutic agents for the treatment of wide variety of malignant diseases such as breast cancer, multiple myeloma, Hodgkin's disease, furthermore, CPA is used as immunosuppressant agent and in organ transplantation, either alone or in combination with corticosteroids [(2)]. Myelosuppression was reported to be a dangerous condition that's related to defect in the blood cell-forming process, which affects body functions of patients, including their quality of life [(3)]. It has been reported that, compensating myelosuppression in chemotherapy is so hard [(4)].

Vitamin B2 (Riboflavin) is a water soluble vitamin [(5)] which found in different food sources. It is well recognized that such vitamin can participate in different redox reactions which is important to human metabolism; furthermore, vitamin B2 is a source for cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) that act as electron carriers [(6)]. Different steps in the oxidation of fatty acids are depending on flavin as electron acceptors in the mitochondria [(7)]. It was found that the effect on oxidation of fatty acids is thought to be responsible for the altered fatty acid profile in hepatic lipids in severely riboflavin-deficient mice which seems to be independent of the dietary source of lipid [(8)]. The effect of riboflavin-deficiency on fatty acid profiles may reflect an overall reduction in the oxidation of fatty acids; while the essential fatty acids are present in the diet accumulate [(9)].

Vitamin B12 is a generic name for a specific group of cobalt-containing corrinoids with important biological activities for humans [(10)]. Vitamin B12 has been reported to be required for the conversion of methylmalonic acid to succinyl-CoA [(11, 12)].

The aim of the study was to evaluate the effects of different doses of vitamin B2 and fixed dose of vitamin B12 on myelosuppression induced by cyclophosphamide in experimental rats.

Materials and Methods

Experimental animals

One hundred healthy adult albino rats of both sexes, three months old, weighing 180-220gm were used in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy, Baghdad University under conditions of controlled temperature. The animals were fed commercial pellets and tap water *ad libitum* throughout the experiment period. The study was approved by the Scientific- and the Ethical- Committees of the College of Pharmacy/University of Baghdad.

Drugs

Cyclophosphamide vial (500 mg) was purchased from Baxter, USA. Vitamin B2 capsule (400 mg) was purchased from Amazing nutrition, USA. Vitamin B12 tablet (1 mg) was purchased from TQ pharma, Japan.

Experimental protocol

The healthy rats were randomly divided into ten groups (10 animals/group) as follows:

Group I: IP injected 1ml/kg/day normal saline for 7 days; this group served as control.

Group II: IP injected with single dose of cyclophosphamide (150 mg/kg).

Group III: Orally-administered vitamin B2 at a dose of (10 mg/kg/day) for 7 days.

Group IV: Orally-administered vitamin B2 at a dose of (40 mg/kg/day) for 7 days.

Group V: Orally-administered vitamin B12 at a dose of (0.1 mg/kg/day) for 7 days.

Group VI: Orally-administered vitamin B2 at a dose (10 mg/kg/day) for 7 days and a single IP injection of (150 mg/kg) of cyclophosphamide at day 7.

Group VII: Orally-administered vitamin B2 at a dose (40 mg/kg/day) for 7 days and a single IP injection of (150 mg/kg) of cyclophosphamide at day 7.

Group VIII: Orally-administered vitamin B12 at a dose (0.1 mg/kg/day) for 7 days and a single IP injection of (150 mg/kg) of cyclophosphamide at day 7.

Group IX: Orally-administered a combination of vitamin B2 at a dose (10 mg/kg/day) and vitamin B12 at a dose of (0.1 mg/kg/day) for 7 days and a single IP injection of (150 mg/kg) of cyclophosphamide at day 7.

Group X: Orally-administered a combination of vitamin B2 at a dose (40 mg/kg/day) and vitamin B12 at a dose of (0.1mg/kg/day) for 7 days and a single IP injection of (150 mg/kg) of cyclophosphamide at day 7.

Twenty-four hour after the end of the treatment duration (i.e. at day 8), rats were euthanized by diethyl ether and then by intra cardiac puncture, 8 ±1 ml of blood was obtained by cardiac puncture for complete blood counts(CBCs) (total WBC, lymphocytes, neutrophils and RBCs).

Bone marrow tissue sample

Femur bone was obtained by making incision in abdomen then extends the incision down to leg, remove skin; soft tissue and connective tissue attached to femur and tibia

then dislocate shift bone and remove tissue attached to it [16].

Bone Marrow Isolation

Isolation of bone marrow was performed according to Sarah R *et al* (2016) [13].

Histological Examination

Bone marrow of each animal was prepared for histological examination according to the method of Junqueira [14, 15].

Statistical Analysis

Data were expressed as the mean values, mean± standard error of the mean (SEM). Unpaired Student t-test was used for testing the significant difference between two groups. The statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for *P*-value less than 0.05.

Results

Table 1 showed that there were non-significant differences *(P*<0.05) in total number of WBCs in the groups of rats orally-administered of different doses of vitamin B2 each alone for one week (**Groups III,** and **IV**) and fixed dose of vitamin B12 for one week (**Group V**) each compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of total number of WBCs were respectively, 7.03 * 10^9/L±0.094, 6.93 * 10^9/L±0.113, 6.97 * 10^9/L±0.115 and 6.99 * 10^9/L±0.113. Furthermore, rats IP injected with CPA at day 7 (**Group II**) produced significant reduction (*P*<0.05) in the total number of WBCs compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of total number of WBCs were respectively, 1.31*10^9/L±0.073 and 6.99*10^9/L±0.113. Moreover, there were significant elevation (*P*<0.05) in total number of WBCs in groups treated with different doses of vitamin B2 each alone for one week (**Groups VI,** and **VII**), vitamin B12 for one week (**Group VIII**), and combination of different doses of vitamin B2 with vitamin B12 (**Group IX** and **Group X**) each for one week prior to IP injection of CPA compared to the corresponding numbers in (**Group II**) rats IP injected with a single dose of CPA. Mean±SEM of total number of WBC were respectively;1.81*10^9/L±0.099, 2.29*10^9/L±0.099, 2.68*10^9/L±0.122, 3.32*10^9/L±0.091, 3.89*10^9/L±0.073, and 1.31*10^9/L±0.073.

Furthermore, table 1 showed that there were significant elevation (*P*<0.05) in total number of WBCs in groups treated with combination of vitamin B2 with vitamin B12 prior to CPA (**Group IX**) and vitamin B2 with vitamin B12 (**Group X**) for one week prior to IP injection of CPA compared to the corresponding total number of WBCs to either use of vitamin B2 or vitamin B12 alone (**Groups VI, VII and VIII**). Mean±SEM of total number of WBCs were respectively, 3.32* 10^9/L±0.091, 3.89* 10^9/L±0.073, 1.81* 10^9/L±0.099, 2.29* 10^9/L±0.099 and 2.68* 10^9/L±0.122.

Table 1 showed that there were non-significant differences *(P*<0.05) in lymphocytes number in the groups of rats orally administered different doses of vitamin B2 each alone for one week (**Groups III,** and **IV**) and vitamin B12 for one week (**Group V**) each compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of lymphocytes number were respectively; 2.05 * 10^9/L±0.016, 2.06 * 10^9/L±0.022, 2.01 * 10^9/L±0.023 and 2.02* 10^9/L±0.02. Furthermore, rats IP injected with CPA at day 7 (**Group II**) produced significant reduction (*P*<0.05) in the lymphocytes number compared to the corresponding number in control (**Group I**) rats. Mean±SEM of lymphocytes number were respectively, 0.14 * 10^9/L±0.016 and 2.02 * 10^9/L±0.02.

Moreover, there were significant elevation (*P*<0.05) in lymphocytes number in groups treated with different doses of vitamin B2 each alone for one week (**Groups VI,** and **VII**), vitamin B12 for one week (**Group VIII**), and combination of vitamin B2 with vitamin B12 (**Group IX**) and vitamin B2 with vitamin B12 (**Group X**) each for one week prior to IP injection of CPA compared to the corresponding numbers in (**Group II**) rats IP injected with a single dose of CPA. Mean±SEM of lymphocytes number were respectively, 0.22 * 10^9/L±0.013, 0.31 * 10^9/L±0.017, 0.39* 10^9/L±0.01,0.48 * 10^9/L±0.013, 0.58 * 10^9/L±0.013, and0.14 * 10^9/L±0.016.

Furthermore, table 1 showed that there were significant elevation (*P*<0.05) in lymphocytes number in groups treated with combination of vitamin B2 with vitamin B12 prior to CPA (**Group IX**) and vitamin B2 with vitamin B12 (**Group X**) for one week prior to IP injection of CPA compared to the corresponding lymphocytes number to either use of vitamin B2 or vitamin B12 alone (**Groups VI, VII and VIII**). Mean±SEM of lymphocytes number were respectively, 0.48 * 10^9/L±0.013, 0.58 * 10^9/L±0.013, 0.22 * 10^9/L±0.013, 0.31 * 10^9/L±0.017and 0.39* 10^9/L±0.01.

Meanwhile, table 1 showed that there were non-significant differences *(P*<0.05) in neutrophils number in the groups of rats orally administered of different doses of vitamin B2 each alone for one week (**Groups III,** and **IV**) and vitamin B12 for one week (**Group V**) each compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of neutrophils number were

respectively, 3.14 * 10^9/L±0.016, 3.13 * 10^9/L±0.021, 3.16 * 10^9/L±0.031 and 3.22 * 10^9/L±0.047.

Furthermore, rats IP injected with CPA at day 7 (**Group II**) produced significant reduction (P<0.05) in the neutrophils number compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of neutrophils number were respectively, 0.51 * 10^9/L±0.048 and 3.22 * 10^9/L±0.047.

Moreover, there were significant elevation (P<0.05) in neutrophils number in groups treated with different doses of vitamin B2 each alone for one week (**Groups VI** and **VII**) and vitamin B12 for one week (**Group VIII**), and combination of vitamin B2 with vitamin B12 (**Group IX** and **Group X**) for one week prior to IP injection of CPA compared to the corresponding numbers in (**Group II**) rats IP injected with a single dose of CPA. Mean±SEM of neutrophils number were respectively; 0.77 * 10^9/L±0.021, 1.01 * 10^9/L±0.023, 1.25 * 10^9/L±0.022, 1.45 * 10^9/L±0.017, 1.76 * 10^9/L±0.034, and 0.51 * 10^9/L±0.048.

Furthermore, table 1 showed that there were significant elevation (P<0.05) in neutrophils number in groups treated with combination of vitamin B2 plus vitamin B12 prior to IP injection of CPA (**Group IX**) and vitamin B2 with vitamin B12 (**Group X**) for one week prior to IP injection of CPA compared to the corresponding neutrophils number to either use of vitamin B2 or vitamin B12 alone (**Groups VI, VII and VIII**). Mean±SEM of neutrophils number were respectively, 1.45 * 10^9/L±0.017, 1.76 * 10^9/L±0.034, 0.77 * 10^9/L±0.021, 1.01 * 10^9/L±0.023 and 1.25 * 10^9/L±0.022.

Table 1 also showed that there were non-significant differences *(P*<0.05) in RBCs number in the groups of rats orally-administered of different doses of vitamin B2 each alone for one week (**Groups III** and **IV**), and vitamin B12 for one week (**Group V**) each compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of RBC numbers were respectively, 4.29 * 10^{12}/L±0.064,4.36 * 10^{12}/L±0.081, 4.41 * 10^{12}/L±0.060 and 4.3 * 10^{12}/L±0.082.

Furthermore, rats IP injected with CPA at day 7 (**Group II**) produced significant reduction (P<0.05) in the RBCs number compared to the corresponding numbers in control (**Group I**) rats. Mean±SEM of RBC numbers were respectively, 2.36 * 10^{12}/L±0.022 and 4.3 * 10^{12}/L±0.082.

Meanwhile, there were significant elevation (P<0.05) in RBCs number in groups of rats treated with different doses of vitamin B2 each alone for one week (**Groups VI,** and **VII**), vitamin B12 for one week (**Group VIII**), and combination of different doses of vitamin B2 with vitamin B12 (**Group IX** and **Group X**) each for one week prior to IP injection of CPA compared to the corresponding numbers in (**Group II**) rats IP injected with a single dose of CPA. Mean±SEM of RBCs numbers were respectively, 2.62 * 10^{12}/L±0.025, 2.92 * 10^{12}/L±0.029, 3.16 * 10^{12}/L±0.034, 3.40 * 10^{12}/L±0.021, 3.70 * 10^{12}/L±0.030, and 2.36 * 10^{12}/L±0.022.

Furthermore, table 1 showed that there were significant elevation (P<0.05) in RBCs numbers in groups treated with combination of vitamin B2 plus vitamin B12 prior to IP injection of CPA (**Group IX**), and vitamin B2 with vitamin B12 (**Group X**) for one week prior to IP injection of CPA compared to the corresponding RBCs numbers to either use of vitamin B2 or vitamin B12 alone (**Groups VI, VII and VIII**). Mean±SEM of RBCs numbers were respectively, 3.40 * 10^{12}/L±0.021, 3.70 * 10^{12}/L±0.030, 2.62 * 10^{12}/L±0.025, 2.92 * 10^{12}/L±0.029 and 3.16 * 10^{12}/L±0.034.

Table 1.Effects of different doses of vitamin B2 and single dose of vitamin B12 each alone and in combination on CBCs (total WBC, Lymphocytes, neutrophils and RBC) after IP injection of cyclophosphamide (CPA) in rats

Group/Treatment	Total number of white blood cells* 10^9/L	Lymphocytes number*10^9/L	neutrophils number*10^9/L	RBC number * 10^{12}/L
Group I/Control (normal saline)	6.99±0.113[a]	2.02±0.02[a]	3.22±0.047[a]	4.3±0.082[a]
Group II/ Cyclophosphamide (150mg/kg)	1.31±0.073[g]	0.14±0.016[g]	0.51±0.048[g]	2.36±0.022[g]
Group III/vitamin B2 (10 mg/kg/day)	7.03±0.094[a]	2.05±0.016[a]	3.14±0.016[a]	4.29±0.064[a]
Group IV/Vitamin B2 (40 mg/kg/day)	6.93±0.113[a]	2.06±0.022[a]	3.13±0.021[a]	4.36±0.081[a]
Group V/Vitamin B12 (0.1 mg/kg/day)	6.97±0.115[a]	2.01±0.023[a]	3.16±0.031[a]	4.41±0.060[a]
Group VI/Vitamin B2 (10 mg/kg/day) with a single IP injection of CPA	1.81±0.099[f]	0.22±0.013[f]	0.77±0.021[f]	2.62±0.025[f]
Group VII/ Vitamin B2 (dose 40 mg/kg/day) and a single IP injection of CPA	2.29±0.099[e]	0.31±0.017[d]	1.01±0.023[e]	2.92±0.029[e]
Group VIII/Vitamin B12 (dose 0.1mg/kg/day) and a single IP injection of CPA	2.68±0.122[d]	0.39±0.01[d]	1.25±0.022[d]	3.16±0.034[d]
Group IX/A combination of vitamin B2 (10 mg/kg/day) and vitamin B12 (0.1 mg/kg/day) and a single IP injection of CPA	3.32±0.091[c]	0.48±0.013[c]	1.45±0.017[c]	3.40±0.021[c]
Group X combination of vitamin B2 (40 mg/kg/day) and vitamin B12 (0.1 mg/kg/day) and a single IP injection of CPA.	3.89±0.073[b]	0.58±0.013[b]	1.76±0.034[b]	3.70±0.030[b]

Each value represents mean ± standard error of means (SEM).
Values expressed in small letters (a, b, c, d, e, f, and g) are significantly different (P<0.05).
Number of animals in each group=10.

Histological examination of rats' bone marrow tissue

Rats IP injected 1ml normal saline (**Group I**, control group), orally-administered different doses of vitamin B2 (**Group III** and **Group IV**), and orally-administered vitamin B12 (**Group V**) each for 7 days shows normal bone marrow section; where, normal appearance of reticular area and leukocyte production area with notice of megakaryocyte are observed in figures (1-A, B, C, D), respectively.

The bone marrow section from (**Group II**) exposed to IP injection of CPA showed massive cell death with adipose tissue, fibroid area distribution and reduction in erythrocyte genesis and leukocyte genesis, with pyknotic nuclei with massive apoptotic cells are observed in figure 1-E.

The bone marrow section from (**Group VI**) orally-administered vitamin B2 for 7 days prior to IP injection of CPA at day seven showed that histological changes include abnormal fibroid area with adipose tissue with pyknotic nuclei with numerous apoptotic cells as shown in figure 1-F.

The bone marrow section from (**Group VII**) orally-administered vitamin B2 for seven days prior to IP injection of CPA at day seven showed that abnormal fibroid area with adipose tissue with pyknotic nuclei with numerous apoptotic cells as shown in figure 1-G.

The bone marrow section from (**Group VIII**) orally-administered vitamin B12 for seven days

prior to IP injection of CPA at day seven showed abnormal fibroid area with adipose tissue with pyknotic nuclei and numerous apoptotic cell as shown in figure 1-H.

However the bone marrow sections from (**Groups IX** and **X**) orally-administered vitamin B12 with different doses of vitamin B2 respectively for 7 days prior to IP injection of CPA at day seven showed mild abnormal fibroid area and pyknotic nuclei with replacement of normal bone marrow area with limited number of apoptotic cells as shown in figures 1-I and 1-J respectively.

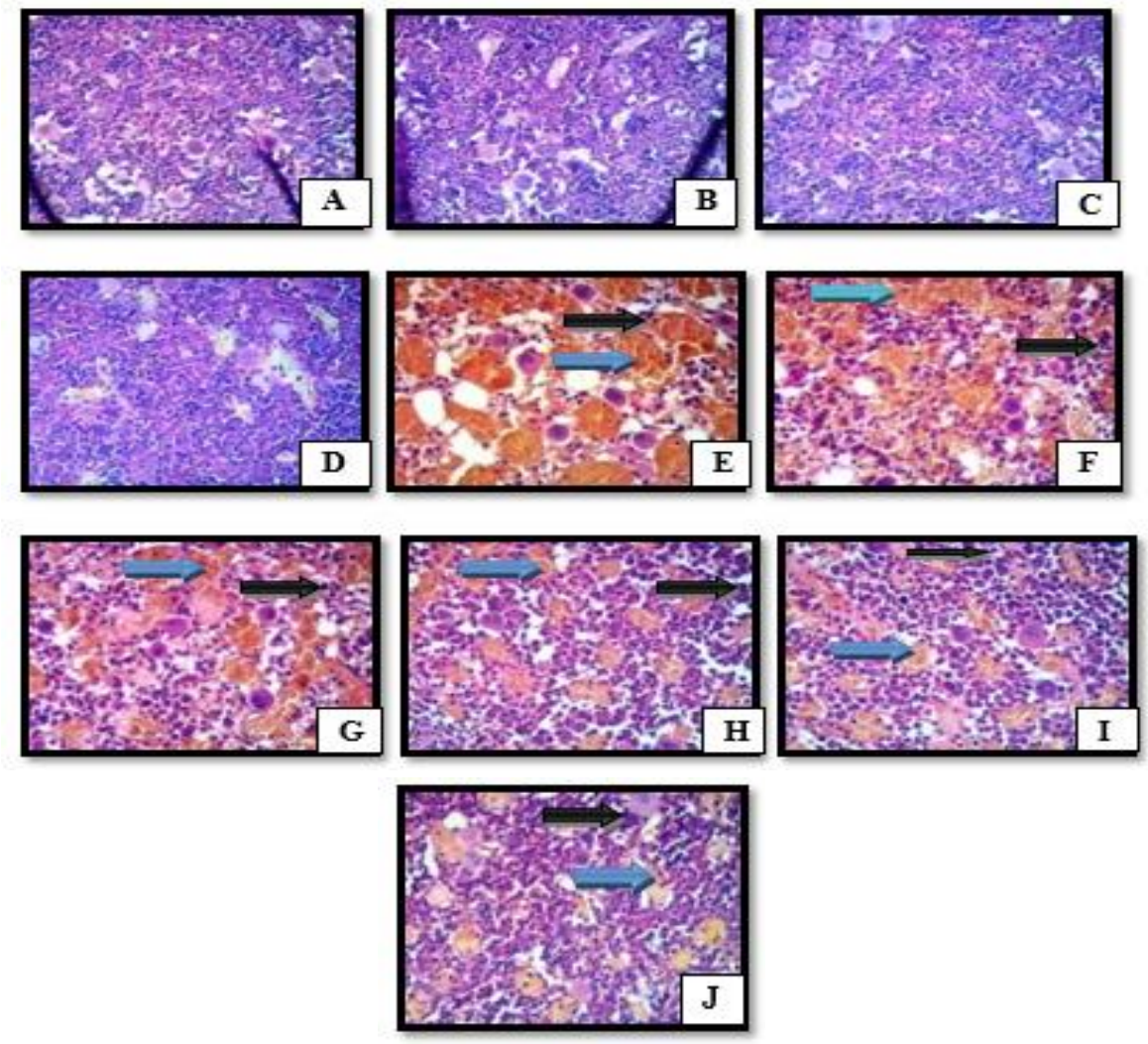

Figure 1. Histopathological section of bone marrow in various experimental rats' groups; (Hematoxylin and eosin; X40).

Discussion

In the present study IP injection of (150 mg/kg) CPA at day 7 (**Group II**) produce significant reduction in CBCs including (total WBCs, lymphocytes, neutrophils, RBCs and Hb) (P<0.05) compared to control rats (**Group I**); authors reported that the myelosuppression induced by CPA occurred due to different mechanisms, which include:

1- Induction of apoptosis: Inappropriate and excessive spontaneous and activation-induced apoptosis can lead to myelosuppression and result in myelodysplasia, thrombocytopenia, leukopenia and lymphopenia [(16)].

2- Induction of hematopoietic stem cell (HSC) senescence: Cells undergo senescence after extensive replication or exposure to a genotoxic or oncogenic stress although senescent cells metabolically active, they are no longer capable of dividing [(17)]. The senescent HSCs induced by CPA have diminished clonogenic activity and express increased levels of SA-β-gal, p16Ink4a and Arf [(18)].

3- Damage to BM stroma has been observed after treatment with CPA [(19)].

In general BM stroma is more resistant to the chemotherapy compared to the effect of chemotherapy on hematopoietic progenitor cells and hematopoietic stem cells; however treatment with CPA can produce damage to BM stroma [(20)].

Results of the current study concerning the effects of CPA on CBCs are coincide with those of Qing-Yu *et al* (2018) who found that CPA induced myelosuppression in mice bone marrow mediated by genotoxic mechanism [(20)]. Recently, Syeda *et al* (2019) also found that CPA can induce myelosuppression via oxidative stress (OS)-mediated DNA damage [(21)]. Similarly, Kartick *et al* (2019) found that CPA-induced myelosuppression and hepatic OS as evident by lipid peroxidation and activity assays of antioxidant enzymes such as SOD [(22)].

Moreover, results of this study shows that -vitamin B2 in dose-dependent manner,-fixed dose of vitamin B12, and -combination of vitamin B2 with vitamin B12 prior to CPA produce a significant increase in CBCs compared to corresponding counts in rats of **Group II** these effects could be explained that riboflavin possess antioxidant property and it considered as an important precursor for FMN and FAD, which served as coenzymes for several enzymes particularly antioxidant enzymes including SOD and catalase [(23)]. Also, riboflavin was reported to play important role in conversion oxidized glutathione (GSSG) to the reduced form (GSH), which plays important role as antioxidant defense factor [(24)]; these roles of riboflavin make it capable to reduce myelosuppression induced by CPA. Furthermore; authors reported that vitamin B12 is also required for the synthesis of methionine and S-adenosyl methionine, which is a common methyl donor required for the maintenance of methylation patterns in DNA that determine gene expression and DNA conformation [(25)]. So that a reduction in the level of vitamin B12 may lead to elevation in DNA damage and alter DNA methylation and elevation in the level of homocysteine [(26)]. Furthermore, Hornung *et al* (2004) explored that vitamin B12 may have myeloprotective effect; where, it played an effective role in patients with rheumatoid arthritis (RA) [(27)].

Effects on rats' bone marrow (BM) histology

In the present study, histopathological examination of BM section of rats IP injected with CPA at a dose (150 mg/kg) at day 7 **(Group II)** confirmed the myelosuppression induced by such drug compared to control **(Group I)** rats; where BM section of CPA-treated rats under light microscope showed massive fibroid tissue replacement of BM with clear vacoulation in addition to distribution of adipose tissue with massive apoptosis were observed figure (1-E). These findings are coinciding with the work of Sun C *et al* (2018) [(28)].

Effect of vitamin B2 (10 and 40 mg/kg) (**Groups VI and VII**) and combination with (0.1 mg/kg) vitamin B12 (**Groups IX and X**), orally-administered prior to CPA showed that there were improvement of the histopathological BM lesions in above-mentioned groups, figures (1-F, 1-G, 1-I and 1-J) compared to (**Group II**) rats (CPA-treated) figure (1-E). In this study, results are in agreement with Zhaoli *et al* (2015); where, a protective effect of vitamin B2 against BM toxicity was observed by histopathological examination [(29)].

Also the effect of (0.1 mg/kg) of vitamin B12 orally-administered prior to CPA (**Group VIII**) showed that there were improvement of the histopathological BM lesions figure (1-H) compared to (**Group II**) (CPA-treated) figure (1-E). In this study, results are in agreement with Demet *et al* (2019); where, a protective effect of vitamin B12 against myelosuppression was observed by histopathological examination [(30)].

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