آیا سایمتدین می‌تواند به عنوان یک داروی محافظ رادیویی و رادیوپروتکتور مطرح باشد؟ مکانیزم عمل پیشنهادی

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چکیده:

شواهد بسیاری وجود دارد که پرتوهای یونساز می‌توانند به ماکرومولکولهای مهم سلول مانند مولکول *DNA* از طریق مکانیزمهای مستقیم و غیرمستقیم آسیب وارد نمائید. استفاده از عوامل شیمیایی برای ایجاد حفاظت نسبی در برابر آسیبهای تشعشعی زمینه مطالعات بسیاری از زمان کشف اثر رادیوپروتکتوری سیستئین در سال ۱۳۴۹ بوده به است. بعضی از داروها چون *MEA*، *AET* و *Wa-2721* عنوان محافظهای رادیویی قوی معرفی شدند اما این ترکیبات باید در دزهای زیاد تجویز شوند و بنابراین اثرهای جانبی گوناگونی را در بردارند. به دلیل گزارشی مبنی بر نقش ایمنومدولاتوری داروی سایمتدین در آزمایشهای متعددی، اثر این دارو بر روی تغییرات لنفوهماتوپوئتیک در دزهای ۸-۱ گری مورد مطالعه قرار گرفت. همچنین اثرات کلاستوژنیک پرتوهای با *LET* بالا و *LET* پائین بر روی سلولهای مغز استخوان با استفاده از روش میکرونوکلئی اسی مورد بررسی قرار گرفت. آزمون میکرونوکلئی یک روش مطمئن و حساس برای تعیین تغییرات ژنتیکی ناشی از عوامل فیزیکی و شیمیایی است. در این مقاله نتایج حاصل از *LET* آزمایشهای متعدد انجام شده برای نشان دادن نقش رادیوپروتکتوری سایمتدین ارائه می‌شود. در تمامی موارد، سایمتدین که یک گیرنده آنتاگونیست هیستامین نوع ۱۵ در *II* می‌باشد، اثرات کلاستوژنیک پرتوهای با *LET* پائین و بالا را با فاکتور کاهش از بدن *(DRF)* ۲-۱/۵ کاهش داده است. این *DRF* با تزریق داخل صفاتی تنها *mg/kg* وزن سایمتدین ۲ ساعت قبل از تابش‌گیری حاصل شده‌است. این دز سایمتدین برای سلولها سمی نیست. روشی که سایمتدین از طریق آن موجب کاهش اثرهای کلاستوژنیک تشعشع می‌شود منظور تبیین این مکانیزم عمل نیز مورد سایمتدین، آرابیتوز ایدسیتوزین و مایتومایسین رادیکالهای آزاد *C* مطالعه قرار گرفت. این داروها از طریق تشکیل در *DNA* موجب ایجاد آسیب بیولوژیکی می‌شود. نتایج نشان می‌دهد که سایمتدین یک اثر آنتی کلاستوژنیک و مهار *p* برابر این داروها دارد. مکانیزم رادیکال اسکاویزی سایمتدین همراه با تقویت سیستم کلوتائین، ترمیم *DNA* و مهار سیتوکروم ۴۵۰ *P* می‌باشد.

کلید واژه‌ها: ۱ـ سایمتدین ۲ـ اثر محافظ رادیویی ۳ـ سلولهای مغز استخوان ۴ـ مکانیسم رادیکال اسکاونجری ۵ـ ترمیم *DNA*

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CAN CIMETIDINE BE USED AS A RADIOPROTECTOR

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ABSTRACT

An in vivo micronucleus assay using mouse bone marrow for identifying radioprotective effect of cimetidine is described. The influence of cimetidine, an antagonist to histamine H2 receptor, on the kinetics of low and high LET radiations such as gamma rays and neutron induced micronuclei as well as the clastogenic effects of chemicals such as benzene, ara C and mitomycin C was tested in Swiss albino male mice. Cimetidine was administered at 15 mg/kg i.p 2 hours prior to irradiation to mice exposed to various doses of gamma rays and neutrons and single dose of drugs. Femoral marrow cells were analysed on slides stained with May-Giemsa. Frequency of micronucleated polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were determined at various time intervals after irradiation. Results obtained indicate that in all cases cimetidine reduced clastogenic effects of both low and high LET radiation by a dose reduction factor (DRF) of 1.5-2. The dose of cimetidine used in these experiments is not toxic for cells. The way in which cimetidine reduces clastogenic effects of radiation might be via radical scavaging mechanism. In order to verify this mechanism of action of cimetidine, protective effects of chemical agents such as benzene, sytosine arabinoside and mitomycin C was also studied. These drugs produce biological effects via free radical formation. Results indicate that cimetidine show an anticlastogenic effect against these drugs. Radical scavanging mechanism of cimetidine is associated by amplification of glutathion system, DNA repair and cytochrome P450 inhibition.

Key Words: *1) Cimetidine* *2) Radioprotective effect* *3) Bone marrow cells* *4) Radical scavanging mechanism* *5) DNA Repair*

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INTRODUCTION

It is well established that ionizing radiation can damage biologically important macromolecules such as DNA via both direct and indirect mechanisms. The use of chemical agents to provide partial protection against radiation injury has been a major field of study over forty years. Since the discovery of radioprotective effect of cysteine in 1949[18], there has been an extensive search for additional compounds that provide radioprotection[24]. Some new drugs such as B-mercaptoethylamine (MEA), cysteamine, aminethylisothrouronium (AET) and WR-2721 have been introduced as potent radioprotectors[3]. In spite of their various side effects, these compounds are effective at high doses. WR 2721, the best of these, is capable of producing DRF about 2.7 for gamma radiation in mice after i.p. injection at doses between 100 - 800 mg/kg[4]. AET, at a dose of 400 mg/kg i.p provides DRF of upto 2.1 as derived from mouse LD50/30 studies[25]. The vast majority of information obtained in the field of radioprotection has been derived from studies using low LET X - and gamma radiation. Exposure to high LET radiation can also be expected during long term space flight, occupational exposure and in tumour radiotherapy. It is shown that neutrons and other high LET radiation induce more severe biological effects than X - and gamma rays for the same absorbed dose[13,23,28,29]. Thus protection against this type of radiation would also be of direct benefit. We have shown that cimetidine is able to produce protection against lymphoid tissue injuries following whole body gamma irradiation at a dose range of 1 - 8 Grays with a dose reduction factor (DRF) greater than 1.5[16]. Cimetidine is an antagonist of histamine type II receptor and is used clinically for teatment of peptic ulcer[2]. It can augment the proliferative as well as cytotoxic responce of lymphocytes. It is also able to prevent interaction of histamine with leukocytes[6,19].

In line with these studies we used cimetidine against low dose gamma and fast neutron induced clastogenic effects in terms of micronuclei (Mn) production. The micronucleus test developed by Schmid and his colleagues[14,21,27] is a sensitive method for assesment of genetic changes induced by chemicals and radiation at very low dosages[12,26]. To verify the mechanism of action of cimetidine on radiation induced clastogenic effects, the protective effect of cimetidine against clastogenic effects of some chemicals such as Benzene, Cytosine arabinoside (ara C) and Mitomycin C (MMC) was also studied.

In the present investigation, kinetics of micronuclei induction in mouse polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) at various post-treatment time intervals at

various radiation and single chemical dose, and the effect of cimetidine on radiation induced micronuclei was studied.

MATERIALS & METHODS

Animals

Swiss male albino mice were purchased from Pasteur Institute, Tehran, Mice were housed in metal mesh cages in good condition and given standard mouse pellet and water ad libitum. All experiments were performed using 4 and 8 weeks old mice.

Treatment

Climetidine: (200 mg/2ml), Chemidarou Co. I.R.Iran), commercially avalilable, was diluted with physiologic serum and injected i.p.at a dose of 15 mg/kg body weight. Mice were treated with a single dose of cimetidine 2 hours prior to irradiation to allow enough time to accumulate in bone marrow.

Cytosine arabinoside (ara C): (Sigma), was diluted in physiologic serum and injected i.p at a dose of 12.5 mg/kg body weight.

Benzene: (Sigma), was diluted in olive oil and injected i.p at a final concentration of 1000 mg/kg body weight.

Mitomycin C: (Sigma), was diluted in physiologic serum and injected i.p at a dose of 2 mg/kg body weight. All drug treated animals were sacrificed 24 hours post-injection.

Irradiation

Gamma rays

Irradiation was carried out using a therapy unit Co-60 gamma ray machine (ACEL, model 780 canada). Both treated and control animals were irradiated at various doses of radiation, 0.25,0.5,0.75, and 1 Gy, in a group of nine in a well ventilated perspex box with a source sample distance (SSD) of 75 Cm at room temperature (24 + /-2 $\mathring{C}$). Dose rate at this condition was 48.7 cGy/min.

Neutrons

An Am - Be neutron source (amersham, U.K) was used for irradiation. Total activity of this source was 10 Curies with a neutron flux of 2.037 x 10 neutrons per second. Energy of neutrons emitted from this source was 4.5 MeV. Dosimetry was performed using a BF3 neutron detector (Amersham, U.K). Mice were irradiated alone or in the presence of cimetidine in a group of nine in a well ventilated perspex box at a dose rate of 0.718 cGy/hr.

Assay Procedures

Mice were sacrificed at various time intervals after irradiation and 24 h after drug treatment. Femoral bone marrow was flushed out with fetal calf serum and cell suspension was prepared. Cells were centrifuged at 1000 rpm for 6 min. Slides were made and left to dry overnight at room temperature, then stained with May Grunwald-Giemsa[21].

Using staining technique of May - Grunwald Giemsa, allow the identification of different anucleate cell types in the bone

marrow. Observation included the number of PCEs and NCEs with and without micronuclei per 1500 total PCE counted per animal. The number of micronuclei essentially represented the number of single micronucleated cells as very few erythrocytes had more than one. For each radiation dose total of 9 animals was used (mean value obtained from three mice for each sampling time). The significance of any intergroup differences in the number of micronucleated PCE and micronucleated NCE, as well as the ratio of PCE to (PCE + NCE) was statistically evaluated by one or two way analysis of variance and student's t-test. Ratio of PCE to (PCE+NCE) was determined for each radiation dose and sampling time to assess radiation and drugs influence on bone marrow proliferation.

RESULTS

* Kinetics of gamma rays induced micronuclei in the presence or absence of cimetidine:

Results obtained in experiments with gamma radiation is summarized in table 1 and graphed in figure 1. The frequency of PCE with micronuclei increased with increasing radiation over doses 0.25 - 1 Gy at all post-irradiation sampling times. The slope of linear regression observed for all post-irradiation intervals was significantly different from zero (Table 1). A statistically significant increase in Mn PCE was observed

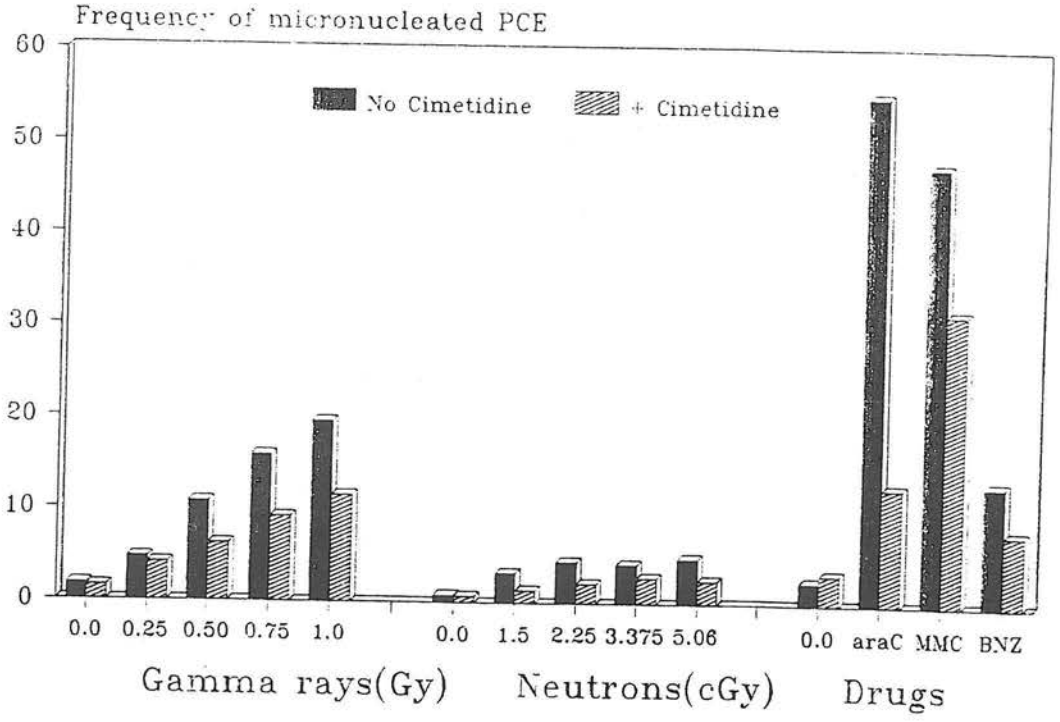

Figure 1: Frequency of MnPCE induced by three different classes of clastogens in the presence or absence of cimetidie

Table 1: Froquency of micronucleated PCEs, NCEs and ratio of PCE/(PCE + NCE) in bone marrow cells of mice exposed to various doses of gamma rays in the presence or absence of 15 mg/kg cimetidine i.p injected 2 h prior to irradiation.

Treatment Gamma Rays (Gy)	*Mean Number of PCE with Mn/1000 PCE*	*Mean Number of NCE with Mn / 1000 NCE*	*Mean Ratio of PCE/(PCE+ NCE)*
0.00	*1.78±0.86*	*4.66±0.97*	*0.39±0.02*
+ Cimet.	*1.55±0.59*	*1.89±0.51*	*0.38±0.03*
	(DRF = 1.67)	*(DRF = 2.47)*	
0.25	*4.66±0.97*	*8.33±2.18*	*0.34±0.05*
+ Cimet.	*4.11±0.97*	*6.00±1.40*	*0.30±0.04*
	(DRF = 1.13)	*(DRF = 1.39)*	
0.50	*10.78±2.67*	*14.0±7.0*	*0.34±0.08*
+ Cimet.	*6.22±2.33*	*12.0±4.89*	*0.37±0.05*
	(DRF = 1.73)	*(DRF = 1.17)*	
0.75	*15.89±2.67*	*32.11±6.86*	*0.33±0.04*
+ Cimet.	*9.22±2.76*	*14.44±5.02*	*0.34±0.06*
	(DRF = 1.72)	*(DRF = 2.22)*	
1.00	*19.56±3.62*	*41.33±7.67*	*0.23±0.07*
+ Cimet.	*11.55±2.91*	*23.00±4.47*	*0.27±0.02*
	(DRF = 1.69)	*(DRF = 1.8)*	

Errors are standard errors of mean values.
Values indicate overall effect of each dose for three sampling times
Values in the bracket shows DRF for each treatment.

at three different sampling times for each radiation dose. These measurements confirm the sensitivity of the test. Resultant pooled data of all sampling times also show a significant difference in the slope of linear regression from zero for radiation doses used in this study with P-value<0.001 (Table 1, Figure 1).

The ferquency of MnNCE also increased with increasing dose of radiation (Table 1). The number of Mn NCE appears to be higher than Mn PCE. This is because the number of NCE found in the field of PCE was much higher than PCEs. A linear dose

responce was also seen for micronulei production in NCEs per 1500 PCEs. Determination of the ratio of PCE / PCE + NCE in gamma irradiated mice showed a pronounced cytotoxic effect of radiation on the bone marrow proliferation (Table 1).

Similar to those recieved radiation alone, groups of mice treated with 15 mg/kg cimetidine 2 hours prior to irradiation. The frequency of MnPCE found in these group were much lower than those treated with radiation alone for all post-irradiation sampling times. There was seen deviation from linearity in the kinetics of micronuclei induction at different sampling times; however, resultant pooled data from mean values obtained for each radiation dose at three sampling times show that the slope of linear regression was statistically significant from zero (P-value<0.001). Regression analysis of results show a slope of 10.11 for regression curve versus 15.60 found for radiation alone. The presence of cimetidine also affected the number of micronuclei found in NCEs. It was much lower than MnNCE seen in those recieved radiation alone. The resultant pooled data of these experiments show a linear dose response which is also statistically significant with P-value<0.001. The DRF calculated for all radiation doses show that cimetidine reduces the frequency of gamma rays induced Mn by a factor of more than 1.5 (Table 1). There is also a linear relationship for proliferation reduction (increase in cytotoxicity) in irradiated mice with P-value<0.001, while in cimetidine treated mice slope of dose response curve is not significant (P-value>0.1). This result shows that cimetidine wiht DRF= 2.6 reduces radiation iduced cytotoxicity.

* Kinetics of neutron induced micronuclei in the presence or absence of cimetidine:

In these experiments although very low dose rate (0.718 cGy/hr) neutrons was used, however dose of 1.5 cGy caused a significant frequency of MnPCE compared to controls (P<0.05). When cimetidine at a dose of 15 mg/kg body weight was injected 2 h prior to neutron irradiation, frequency of MnPCE greately reduced by a DRF of 1.5 -2.3 (Table 2, figure 1). The presence of cimetidine also affected the number of Mn found in NCEs but analysis of variance did not show a significant difference for Mn induced by various doses of neutrons. Proliferation of bone marrow cells was not affected by the presence of cimetidine (Table 2). The values calculated for the ratio of PCE/(PCE+NCE) was quite similar to those mice recieved neutron alone.

TABLE 2: Frequency of micronucleated PCEs, NCEs and ratio of PCE/(PCE+NCE) in bone marrow cells of mice exposed to various doses of neutrons in the presence or absence of 15 mg/kg cimetidine i.p injected 2 h prior to irradiation.

Treatment Neutrons (Gy)	*Mean Number of PCE with Mn/1000 PCE*	*Mean Number of NCE with Mn / 1000 NCE*	*Mean Ratio of PCE/(PCE+ NCE)*
0.00	*0.78±0.53*	*0.89±0.30*	*0.50±0.01*
+ Cimet.	*0.67±0.33*	*0.55±0.41*	*0.50±0.018*
	(DRF = 1.16)	*(DRF = 1.60)*	
1.50	*3.22±0.44*	*2.00±0.41*	*0.53±0.03*
+ Cimet.	*1.33±0.41*	*1.22±0.41*	*0.55±0.03*
	(DRF= 2.42)	*(DRF=1.64)*	
2.25	*4.44±0.51*	*2.55±0.55*	*0.53±0.03*
+ Cimet.	*2.11±0.50*	*1.67±0.60*	*0.55±0.02*
	(DRF = 2.10)	*(DRF = 1.53)*	
3.375	*4.22±0.60*	*1.86±0.50*	*0.50±0.03*
+ Cimet.	*2.78±0.51*	*1.22±0.33*	*0.50±0.02*
	(DRF = 1.52)	*(DRF = 1.52)*	
5.06	*4.89±0.90*	*1.44±0.51*	*0.50±0.02*
+ Cimet.	*2.44±0.33*	*1.01±0.44*	*0.53±0.03*
	(DRF = 2.0)	*(DRF = 1.43)*	

Errors are standard errors of mean values.
Values indicate overall effect of each dose for three sampling times.
Values in the bracket shows DRF for each treatment.

* Effects of cimetidine on micronuclei induce by Benzene, ara C and MMC:

Table 3 and figure 1 shows the results obtained for treatments with benzene. ara C and MMC, Ara C at adose of 12.5 mg/kg body weight caused a high frequency of micronuclei in PCEs after 24 h post injection. The frequency of MnPCE was found to be 55.2 per 1500 PCEs. Injection of cimetidine at a dose of 15 mg/kg 2 h prior to ara C treatment reduced the frequency of MnPCE to 12.6. DRF calculated show a

4.38 fold reduction in clastogenic effect of ara C (Table 3).

TABLE 3: Frequency of micronucleated PCEs, NCEs and ratio of PCE/(PCE+NCE) in bone marrow cells of mice treated with 12.5 mg/kg ara C, 2 mg/kg Mitomycin C and 1000 mg/kg benzene in the presence or absence of 15 mg/kg cimetidine.

Treatment	*Mean Number of PCE with Mn/1000 PCE*	*Mean Number of NCE with Mn / 1000 NCE*	*Mean Ratio of PCE/(PCE+ NCE)*
0.00	*2.40±0.60*	*2.20±0.37*	*0.52±0.01*
+ Cimet.	*3.20±0.86*	*1.40±0.50*	*0.48±0.02*
ara C	*55.20±6.93*	*4.20±1.39*	*0.42±0.05*
+ Cimet.	*12.60±1.16*	*2.40±0.68*	*0.48±0.02*
	(DRF = 4.38)	*(DRF = 1.75)*	
Mitomycin	*47.60±1.28*	*2.20±0.37*	*0.53±0.02*
+ Cimet.	*31.60±4.64*	*2.40±0.32*	*0.47±0.03*
	(DRF = 1.51)	*(DRF = 0.92)*	
Benzene	*13.00±1.89*	*3.00±0.13*	*0.50±0.03*
+ Cimet.	*7.80±1.59*	*3.40±0.81*	*0.44±0.03*
	(DRF = 1.70)	*(DRF = 0.90)*	

Errors are standard errors of mean values.
Values are mean values of 3 mice at 24 hours sampling time.
values in the bracket shows DRF for each treatment.

Mitomycin C at 2 mg/kg also induced high frequency of MnPCE. The number of MnPCE scored was 47.6 per 1500 PCEs after 24 hours i.p injection. Although injection of cimetidine 2 h prior to MMC treatment caused a reduction in MnPCE induction but the reduction was not as high as it was observed for ara C. The DRF calulated was about 1.5 for MMC. Similar observation was made for benzene treatment. Benzene at a dose of 1000 mg/kg body wight induced only 13 MnPCE per 1500 PCEs and treatment with cimetidine reduced this frequency to 7.8; therefore DRF calculated was about 1.7 (Table 3).

DISCUSSION

The micronucleus test is a reliable effective alternative for the evaluation of clastogenic effects of physical and chemical agents[10].

Redioprotective effects of cimetidine is shown in tables 1-3 and figure 1. Cimetidine is shown to reduce effectively the number of radiation induced micronuclei both in PCEs and NCEs at a low dosage used in these experiments (15 mg/kg body weight). As it is shown in table 1 and 2, cimetidine reduces the overall clastogenic effects of gamma rays by a factor of greater than 1.5 and produces a DRF of 1.5 - 2.3 for neutron irradiation. This finding is consistent with our observations for the effect of cimetidine on lymphocyte production centres on whole body irradiated CD-1 mice at doses from 1 to 8 Gy[16]. Results obtained with cimetidine in the present investigation is similar to the results of Garriot and Growe[5] who found a reduction in micronuclei production in mouse bone marrow cells for AET at dose of 0 - 2.5 Gy gamma rays. Although these authors used much higher doses of AET for producting radiation protection (300 mg/kg). AET is known as a potent radioprotector[25]. Thiol compounds are th. most powerful class of protective agents and several mechanism of protection (e.g. hypoxia, radical scavanger hypothesis, mixed disulphide hypothesis, and mitotic inhibition) have been postulated [for detailed review see[1,15]].

Radiation chemical studies have shown that free radicals are primarily responsible for the indirect effects of radiation and production of hydrogen peroxides. In the normal bone marrow cells, kidney and liver of mammals some enzymes such as glutathion reductase and catalase are synthetised normally which have a role in hydrogen peroxide catalysis[17]. Ilyinskikh and Ilyinskikh (1988)[11] showed that inhibition of the activity of these enzymes (glutathion reductase and catalase) leads to an increase in micronuclei produc- tion. It seems that CD4 + lymphocytes (effector T cells) have a role in induction of glutathion redutase and catalase enzymes. A great deal of evidence implicates that histamine is the primary cause for several of the acute physiological responses, including the finding that elevation of plasma histamine level in monkeys after exposure to radiation and release of histamine from arteries[9]. In case histamine is released from arteries, then T suppressor cells are activated and the activity of CD4+ cells would be inhibited. It is shown that cimetidine an antagonist of histamine type II receptor, not only inhibits the suppressor T cells, but also is able to inhibit the activity of T suppressor cells activity[20]. On the other hand, Gifford and his colleguses (1980b, 1983)[5,7] have shown that cimetidine augment the proliferative capactity of lymphocytic cells.

These investigations indicate that, administration of cimetidine before irradiation leads to the inhibition of T suppressor cells and increases the proliferation of CD4+ lymphocytes. This causes production of glutathion reductase and catalase enzyme which prevent DNA damage and eventually reduces clastogenic effects of radiation. Reduction of the frequency of micronuclei observed in the present investigation (Tables 1-3 and Figure 1) might be due to the effects of cimetidine at cellular level. We, therefore, postulate that cimetidine reduces clastogenic effects of radiation via a radical scavanging mechanism through enzyme catalysis. This proposal was examined using benzene which affect DNA double strand via free radical induction and drugs such as cytosine arabinoside and mitomycin C which damage DNA indirectly. It was observed that cimetidine also can protect bone marrow cells against clastogenic effects of these chemicals. This work shed more light on the idea of radical scavanging mechanism of cimetidine. further investigation is needed to verify radioprotective ability of cimetidine, this widely used and available drug.

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