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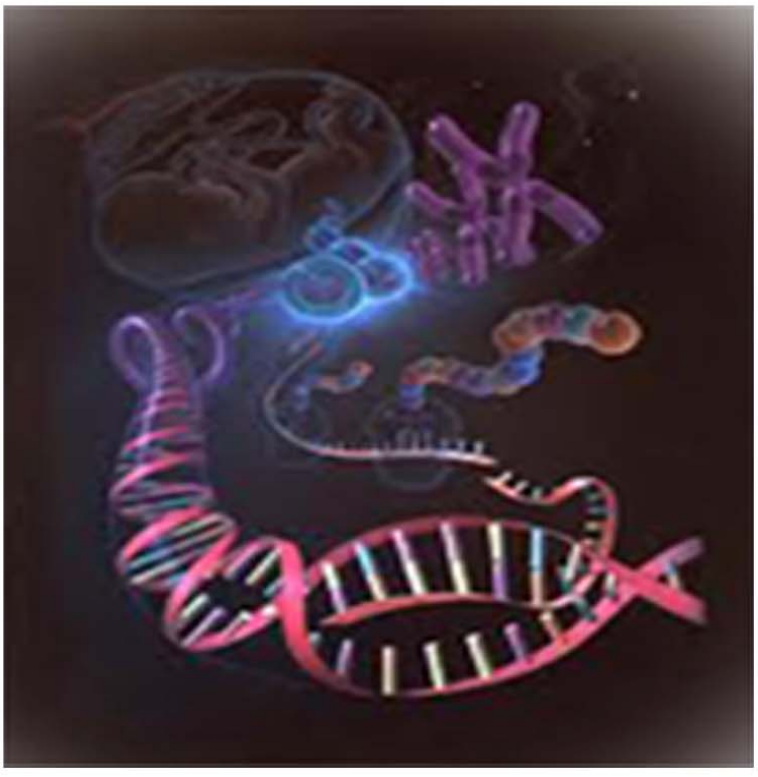

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Research Paper

EFFECT OF QUERCETIN ON MICRONUCLEUS STUDY IN MICE

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The present investigation planned for the possible chemoprotective activity of orally administered quercetin against Cyclophasphamide-induced cyto- and genotoxicity towards mouse somatic cells in vivo. DNA strand breaks, micronuclei formation, and mitotic activity were undertaken in the current study as markers of cyto- and genotoxicity. The frequency of micronucleated polychromatic erythrocytes (MNPCEs) and the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCE/NCE) were also evaluated. The micronucleus assay revealed that all doses of the extract tested presented no genotoxic activity; in addition, the two highest doses reduced the MNPCEs and increased the PCE/NCE ratio in the bone marrow and restored. The biochemical and cytogenetic determinations were carried out 24 h after CYP injection. The results of the present study suggested that the quercetin and have a protective effect against CYP-induced oxidative stress and genotoxicity. Quercetin was neither cytotoxic nor genotoxic in mice at doses tested. Pretreatment of mice with quercetin significantly reduced Cyclophasphamide -induced genotoxicity and cytotoxicity in bone marrow cells, and these effects were dose dependent. Moreover, prior administration of quercetin ahead of Cyclophasphamide challenge ameliorated oxidative stress markers. In conclusion, Quercetin has a protective role in the abatement of Cyclophasphamide -induced cyto and genotoxicity in mice the bone marrow cells. Based on these studies Quercetin could be an ideal antioxidant derivative drug to decrease the Cyclophasphamide -induced bone marrow suppression in anti-cancer therapy.

Keywords: Quercetin, Cyclophasphamide, Anti-cancer therapy, Genotoxicity, Cytotoxicity MNPCEs, PCE/NCE

INTRODUCTION

Camptothecin, a pentacyclic alkaloid originally isolated from the Chinese plant *Camptotheca acuminata* (Wall and Wani, 1996), is one of the most important lead compounds in anticancer research. The antitumor activity of camptothecin

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is thought to be due to its ability to stabilize the reversible covalent DNA topoisomerase I complex (Hartmann and Lipp, 2006; Pommier, 2006), preventing the relegation step of the breakage/ rejoining reaction mediated by the enzyme. The net result is that the drug causes fragmentation of chromosomal DNA, cell death, extensive sister chromatid exchange, and chromosomal aberrations (Backer *et al*., 1990; Sortibŕan *et al*., 2006).

After application of topoisomerase I inhibitors, damage to DNA may result as DNA fragmentation, chromosomal breaks, and micronuclei (MN) formation causing genotoxicity and may lead to carcinogenesis.

Experimental observations have shown that some flavonoids are able to enhance the cytotoxic action of the chemotherapeutic drugs without damaging normal cells (Liesveld *et al*., 2003). Consistent with these notions, quercetin, a polyphenolic compound widely distributed in food of plant origin, has been reported to have antitumor effects against several cancer cells (Scambia *et al*., 1990; Scambia *et al*., 1992). The antitumor effects of quercetin have been reported to induce cell growth inhibition and of *G. Scambiaptosis* in variety of tumor cells (Choi *et al*., 2001).

Quercetin has also been shown *in vitro* to increase the concentration of DNA topoisomerase II inhibitors, doxorubicin, and daunorubicin in some multidrug-resistant cancer cell lines (Cai *et al*., 2005). Moreover, previous reports demonstrated that quercetin increases the growth inhibitory effect of TPT in the treatment of human breast cancer cells (Akbas *et al*., 2005). Enhancements of oral bioavailability and reducing gastrointestinal toxicity of irinote can by quercetin have been also reported (Attia, 2008). The ability of Quercetin to improve the therapeutic outcome on the scoring of MN, and mitotic activity were undertaken in the present study as markers of cyto- and genotoxicity analysis.

MATERIALS AND METHODS

Adult male Swiss albino mice weighing 25-30 g (10-12 weeks old) were approved by IAEC for animal experiment. The animals were maintained under standard conditions of humidity, temperature (25 ± 2%C), and light (12-h light/12-h dark). They were fed with a standard mice pellet diet and had free access to water.

Quercetin (HIMEDIA labs – Mumbai, India) was administered by gavage in propylene glycol as a vehicle. Gavages administrations were made 24 h and 1 h prior to the Cyclophasphamide intraperitoneal injection. Control animals were given propylene glycol vehicle only. Quercetin was administered at the doses level of 50 and 100 mg/kg. Upon conversion of animal dose to the equivalent human dose, a dose of 100 mg/kg quercetin in mice was found to be corresponding to 8.1 mg/kg in humans.

Accordingly, for an average person weighing 60 kg, 486 mg quercetin would be needed. National dietary record-based cohort assessments of the intake of quercetin from the habitual diet indicated daily levels of quercetin as high as 200-500 mg may be attained by high-end consumers of fruits and vegetables, especially in cases where the individuals consume the peel portion of quercetin-rich fruits and vegetables, such as tomatoes, apples, and onions (USDA, 2006).

Cyclophosphamide (HIMEDIA labs – Mumbai,

India) was dissolved in saline immediately before use. All other chemicals were of the finest analytical grade.

Experimental Protocol: Male mice were acclimatized for 2 days and divided into 4 groups consisting of 5 mice each, set up as follows: Group 1: mice were served as a control group and treated daily with the vehicle only for two consecutive days; Group 2: mice were injected with a single dose of 50 mg/kg Cyclophosphamide alone Groups 3 and 4: mice were treated with quercetin in a dose of 50 or 100 mg/kg, respectively, once a day, for two consecutive days and the last day CYP induction 1 h prior to cell harvest.

Bone Marrow Micronucleus Test: The remaining femora from the same animals used for the estimation of MN frequencies and mitotic activity. Bone marrow smears were done, and the slides were stained with May-Gruenwald/ Giemsa solutions as described earlier [SM Attia-2007]. Per animal, 1,000 polychromatic erythrocytes (PCE) were randomly scored microscopically for the presence of MN. In addition, the number of PCEs among 1,000 normochromatic erythrocytes (NCE) per animal was recorded to evaluate bone marrow suppression, mitotic activity was calculated as %PCE = [PCE/(PCE + NCE)] × 100.

Statistical Analysis: Data were expressed as the mean ± standard deviation (SD) of the means. The analyzed parameters were tested for homogeneity of variance and normality and were found to be normally distributed.

The data were, therefore, analyzed by employing nonparametric tests, Mann-Whitney *U*-test, ANOVA, followed by Tukey-Kramer for multiple comparisons. Results were considered significantly different if the *P* value was < 0.05.

RESULTS

Effect of Quercetin on Cyclophosphamide – Induced MNPCE and Bone Marrow Suppression

The results of the micronucleus test are presented in Table 2. The frequency of MNPCE in the positive control mutagen cyclophosphamide was significantly higher when compared to the solvent control group ($P < 0.02$). Similarly, Cyclophosphamide at a single dose of 0.5 significantly increased the frequency of MNPCE ($P < 0.01$).

Moreover, the mitotic index was significantly decreased after treatment with Cyclophosphamide compared to the solvent control group. Pretreatment with quercetin was found to significantly decrease the frequency of MNPCE especially at the higher dose of quercetin as compared to the values obtained after treatment with Cyclophosphamide alone. The reduction of mitotic index induced by CYP was found to be restored by pretreatment with the higher dose of quercetin (Tables 1 and 2).

The anticlastogenic effects of pretreatment with quercetin on the frequency of MNPCEs and the PCE/NCE ratio are shown in the Figure. There was a significantly greater frequency of MNPCEs in CYP-treated rats (group 2) compared with the control (group 1) and the groups given only extract (groups 3, 4) ($P < 0.01$). Figures 1, 2 and 3 are showing normal distribution of PCE, NCE and MN.

Furthermore, the ratio of PCEs to NCEs, as one index of cytotoxicity, was decreased in animals treated with CYP. However, in animals pretreated with quercetin, the PCE/NCE ratio was

Table 1: Micro Nucleated PolyChromatic Erythrocytes in 1000 PCEs

	Normal	Cyclophosphamide	Quercetin (50 mg)	Quercetin (100 mg)
Min to Max	8-10	74-79	12-18	20-24
Mean	8	76	16	22
SD	1.02*	0.80*#	0.64#	1.04#
Statistical Significance*	*NC vs CYP0.002		# CYP vs QE0.001	

Table 2: Frequency of MNPCE and Mitotic Activity (% PCE) in Bone Marrow of Mice Treated With Quercetin and Cyclophosphamide (Mean ± SD)

Treatment groups (mg/kg)	%MNPCE (mean ± SD)	%PCE (mean ± SD)
Control	0.34±0.12	46.4±1.72
Quercetin (50) +CYP	0.32±0.08	48.2 ± 2.20
Quercetin (100) +CYP	0.30±0.12	46.4 ± 1.81
Cyclophosphamide (50)	2.04±0.22#	42.4 ± 2.46#

Figure 1: Normal Distribution of PCE and NCE in Rat bone Marrow

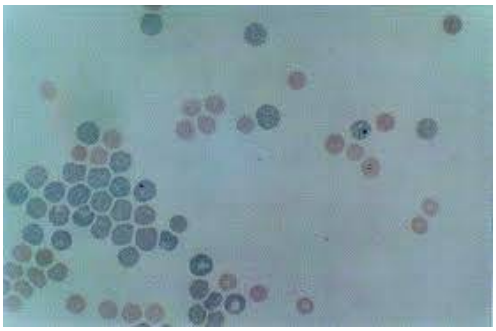


Figure 2: Normal distribution of PCE and NCE in Rat bone Marrow and MN -Micronucleus

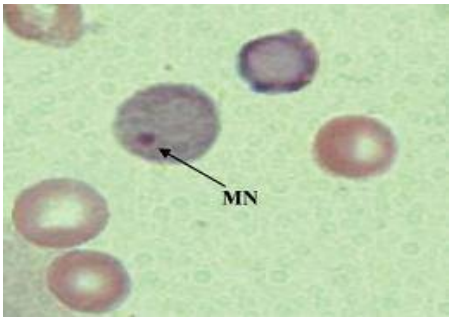


Figure 3: PCE (P) and NCE (N) - in Rat Bone Marrow (Normal Control)

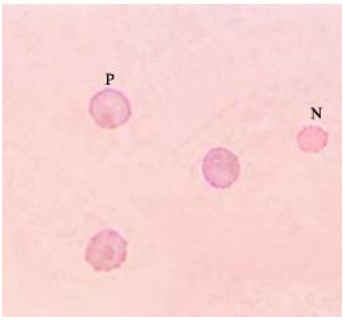
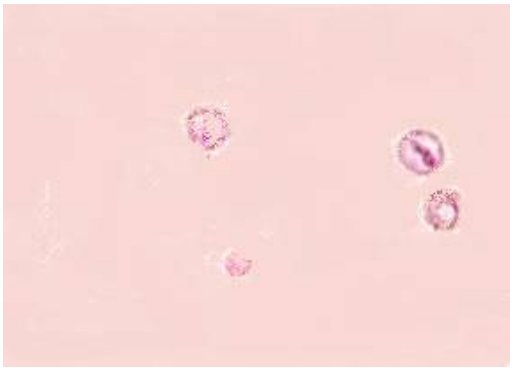


Figure 4: PCE and NCE (Cyclophosphamide)



increased significantly ($P < 0.05$) compared to the CYP group (group 2) (Figure 4).

Pretreatment with Quercetin 50 and 100 mg/kg b.w. resulted in a MNPCE frequency of 22 ± 1.04 respectively, which was significantly ($P < 0.001$) less than the rate of 76 ± 0.80 observed with CYP treatment.

#$P < 0.01$ versus control (Mann-Whitney *U* test)

DISCUSSION

The present investigation is to determine whether nontoxic doses of the bioflavonoid quercetin, a strong antioxidant present in the human diet, have influence on the cyto- and genotoxicity induced by the anticancer topoisomerase-I inhibitor, Cyclophosphamide, on mice bone marrow cells *in vivo*. The positive control mutagen cyclophosphamide was used in this study, and this compound produced the expected responses. The results of cyclophosphamide were in the same range as those of the earlier studies (Attia *et al*. 2010; Papiez *et al*., 2008). These data confirmed the sensitivity of the experimental protocol followed in the detection of DNA damaging effects.

In this study frequency of MNPCE in bone marrow of mice treated with quercetin (100 mg) were 0.30±0.12 when compared to cyclophosphamide 2.04 ± 0.22 ($P < 0.01$) (Table 2)

Micro Nucleated PolyChromatic Erythrocytes in 1000 PCEs with quercetin (100 mg) were 20 – 24 ± 1.04 when compared to cyclophosphamide 74-79 ± 0.80 ($P < 0.001$) respectively (Table 2).

The present study reveals that quercetin was neither cytotoxic nor genotoxic at the doses tested. Moreover, it is able to protect mouse bone marrow cells against the Cyclophosphamide induced cyto- and genotoxicity. These results corroborate earlier studies, where oral administration of quercetin did not cause DNA damage in the bone marrow cells (Papiez *et al.*, 2008; Attia *et al*., 2009). In fact, the ability of quercetin to confer marked protection against different toxic chemical agents has been described. Quercetin mediated inhibition of bacterial mutagenicity induced by different mutagens [17&18] and mouse clastogenicity induced by the chemotherapeutic agent; cisplatin has been reported (Papiez et al., 2008).

Quercetin is known as a potent free radical scavenger, capable of inhibiting lipid peroxidation in *in vitro* and *in vivo* systems (Attia, 2010; Attia *et al*., 2009; Fiorani *et al*., 2001).

It has been reported that TPT induces a decrease in the antioxidant enzyme activities in healthy rabbit liver [19&20]. This can induce cyto- and genotoxicity through the failure of the antioxidant defense mechanisms, since antioxidants are able to protect non tumor cells acting as anti-genotoxins without compromising the anti-neoplastic effects.

The increased GSH and GSH/GSSG levels suggest that protection by quercetin may be mediated through the modulation of cellular antioxidant levels. These observations confirm earlier studies in which quercetin was reported to elevate GSH, glutathione peroxidise and superoxide dismutase and to reduce lipid peroxidation (Papiez *et al*., 2008; Attia *et al*., 2009).

Quercetin was effective in reducing cyto- and genotoxicity induced by Cyclophosphamide in bone marrow cells and may possibly decrease the risk of secondary tumors in cells that were not originally neoplastic.

The inhibition of micronucleus formation by the quercetin suggests that the antimutagenic potential of the quercetin is most probably due to antioxidant and anticarcinogenic activity related to the high antioxidant capacity of the quercetin.

CONCLUSION

The Protective effect of quercetin could be possibly described to its radical scavenger effect that modulated the changes induced by Cyclophosphamide. Based on the data presented here, strategies can be developed to decrease the deleterious effects of Cyclophosphamide in normal cells by using quercetin.

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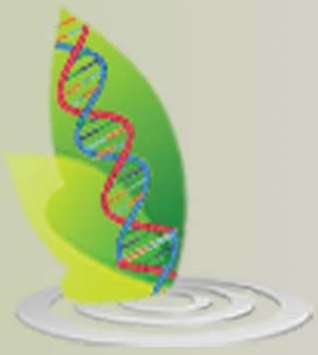

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