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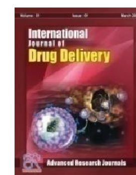

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Role of Quercetin in chemoprevention against wide range of carcinogens and mutagens

Savithiri Shivakumar[1,2], Yasodha Lakshmi Tadakaluru[1], Raja Ratna Reddy Yakkanti[2,3], Sannidhi Ranga Suresh[3], Chandra Sekhar Pasula*[1,2]

*Corresponding author:

Chandra Sekhar Pasula

[1]Palamur Biosciences Private Limited, SVS Medical College Campus, Mahabubnagar-509001, Telangana, India.
[2]School of Life Sciences, Manipal University, Manipal 576104, Karnataka, India.
[3]Central Research Laboratory, SVS Medical College Campus, Mahabubnagar-509001, Telangana, India.

Abstract

Quercetin is a ubiquitous plant flavoniod with significant pharmacological and clinical activity. In this study we determined to demonstrate the protective role of quercetin against range of mutagens and carcinogens in a combination of *in vitro* and *in vivo* studies via different mechanisms. Quercetin demonstrated significant protective role against sodium azide, benzo(a)pyrene, cyclophosphamide monohydrate, methyl methane sulphonate and etoposide compared to other mutagens. Quercetin is effective in both *in vitro* and *in vivo* test conditions and also in the presence as well as in the absence of metabolic activation system (Rat liver S9). Auto oxidation, antioxidant properties, inhibition of pro-mutagens metabolism by CYP1A activity and multiple antimutagenic and adaptive responses, mechanisms of quercetin may account for its protective role in cancer prevention. In conclusion, the results clearly indicate that quercetin plays a significant role against mutagens that act by direct DNA binding (form DNA adducts), pro-mutagens and alkylating agents with free radical generation; which could be the rationale for its potent anticancer activity against particular cancer types.

Keywords: Quercetin, adaptive response, cancer, Ames test, chromosomal aberration, cell gene mutation

Introduction

Cancer is the second leading cause of death with significant mortality and morbidity throughout the world. Though molecular based disease specific cancer therapy is available, strive for an ideal chemo preventive/therapeutic candidate continues. There is increasing evidence that bioactive compounds with antimutagenic activity are known to protect cellular components from genotoxic damage and prevent diseases. Quercetin is a natural flavonoid abundantly present in plant foods. It has significant pharmacological and medicinal properties including antioxidant, anti-inflammatory, antimutagenic and anticancer activity [1].

Its wide clinical activity may suitably correlates with its pleotropic activity in target cells/tissues. Quercetin has been shown to possess anti tumour effects on several malignant leukemia cell lines such as HL-60, K562, NB4 and Jurkat cells and its clinical utility as an anticancer drug has been investigated widely [2,3]. In several combination studies of quercetin with natural compounds and chemotherapy drugs has resulted synergistic effects though the exact anticancer mechanism for clinical efficacy is largely unclear [4, 5, 6]. However it shows strong antioxidant activity and a significant antimutagenic effect was also demonstrated in combination with ascorbic acid [7].

Adaptive response (AR) is a phenomenon, when cells are exposed to the lower non mutagenic concentrations of quercetin will become resistant to subsequent doses of other mutagens or carcinogens. There is limited data available on the adaptive response of quercetin, i.e. Chromosomal aberration test with cultured V79 cells [8] and micronucleus test in human peripheral lymphocytes [9] are the little evidence to demonstrate. Therefore an inclusive assessment in varied test systems with range of mutages/carcinoges is required for complete understanding of the mechanism. For the demonstration of quercetin mode of action in antimutagenicity and adaptive response, a wide range of mutagens/carcinogens were selected (Table-1), based on their mutagenic properties in varied test systems, mode of action in cancer development and clinical significance.

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Table 1: Selected chemical mutagens and their properties

Mutagen	IARC category	Mode of action (in mutagen city)	Distribution/Therapeutic importance
Benzo (a) pyrene (BP)	1	Direct binds to DNA, form DNA-BP adduct	Environment and food mutagen
Cyclophosphamide monohydrate (CP)	1	Alkylating agent: add alkyl group to DNA and form free radicals	Anti cancer drug
Mitomycin C (MMC)	2B	DNA cross linking agent	Food mutagen
Methyl methane sulphonate (MMS)	2A	Alkylating agent	Environment mutagen
Etoposide (Et)	1	Chromosomal mutation and topoisomerase II inhibitor	Anti cancer drug
Hydralazine (Hy)	3	DNA damage – point mutations	Muscle relaxant (Essential medicine)
Bendamustine (BH) hydrochloride	-	Alkylating agent	Anti cancer drug
2 Nitrofluorine (2NF)	2B	Point mutations to DNA	Reference mutagens
Sodium azide (SA)	-	Point mutations to DNA	
9 Aminoacridine (9AA)	-	Point mutations to DNA	
2 Amino anthracene (2AA)	-	Indirect mutagen forms DNA adduct	

Because a strong correlation exists between genetic damage and carcinogenesis, identifying the natural compounds with antimutagenic activity could be beneficial in cancer treatment. Consequently we determined to investigate antimutagenic activity/adaptive response of quercetin using a combination of *in vitro* and *in vivo* genetoxicity testing standard test battery. These tests are routinely employed for regulatory compliance of new chemical entities (NCE) in preclinical drug discovery programme. The Organisation for Economic Co-operation and Development (OECD) guidelines and the International Workshops on Genotoxicity Testing (IWGT) recommended protocols were followed for these tests [10-13]. These tests include: bacterial reverse mutation (Ames) test using histidine dependent *Salmonella* test strains i.e. TA 1537, TA 98, TA 100 and TA 102, chromosomal aberration test (CAT) using CHO K1 cell line to detect structural chromosome aberrations, cell gene mutation assay (CGM) using L5178Y TK+/- cells for the detection of mutagenicity using eukaryotic cell line and bone marrow micronucleus test in mice for clastogencity evaluation of test chemicals, *in vivo*.

It is also extremely important to realize that the main focus of these antimutagenicity tests should be towards understanding of the mode of action of the compounds and the ability to correlate short term results with anticarcinogenicity experiments in animal models [14].

Materials and Methods

Chemicals and culture media

Quercetin (6151-25-3), 9-aminoacridine (90-45-9), sodium azide (26628-22-8), 2-nitrofluorene (607-57-8), hydralazine (304-20-1), etoposide (33419-42-0), methyl methane sulphonate (66-27-3), mitomycin-C (50-07-7), 2-aminoanthracene (613-13-8), benzo(a)pyrene (50-32-8), bendamustine hydrochloride (3543-75-7), cyclophosphamide monohydrate (6055-19-2), magnesium sulphate($MgSO_4.7H_2O$), citric acid monohydrate, potassium phosphate dibasic (K_2HPO_4), sodium ammonium phosphate, ($NaHNH_4PO_4.4H_2O$), dextrose, L-histidine, D- biotin, sodium chloride, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), D- glucose -6- phosphate, β NADP, magnesium chloride, dimethyl sulphoxide, potassium chloride, RPMI-1640 medium, Hams F12 K medium, trifluro thymidine (TFT), KCl, KH_2PO_4, NaOH, $NaHCO_3$, giemsa stain, trypan blue and colchicine were obtained from sigma. Fetal bovine serum (FBS), antibiotic solution (penicillin/streptomycin, amphotercin-B), Dulbecco phosphate buffer saline (DPBS) ampicillin, tetracycline, nutrient agar from himedia. Agar (BD falcon), nutrient broth (oxoid), crystal violet, glacial acetic acid, methanol (Merck) [15].

Metabolic activation system (Rat liver S9 fraction)

In vitro assays were conducted both in the presence (+S9) and absence (-S9) of metabolic activation system. These test systems does not contain the enzyme systems which are generally present in mammals to metabolize pro-mutagens to active electrophilic metabolites able to react with DNA. Sometimes, these pro-mutagens act together with a mammalian enzyme system and can

produce mutagenic metabolites. So, it is necessary to enrich with external metabolic activation system that is post mitochondrial fraction (S9) [15]. Rat liver S9 fraction was obtained from Molecular Toxicology, NC 28607, USA and stored at -80 C. S9 fraction was supplemented with essential cofactor mixture and used at a final concentration of 10% v/v (Ames) and 2% v/v (cell gene mutation and chromosomal aberration test) [16].

Preparation of drug

Lower non cytotoxic and non mutagenic concentrations of quercetin was selected for antimutagenicity /adaptive response, whereas the mutagenicity of quercetin and reference standards selected for the test has been demonstrated in author's laboratory for dose selection.

A stock solution of quercetin and reference mutagens was prepared in DMSO and stored at -20^{0}C. The varying concentrations of quercetin was used in Ames test (0.5mg/plate), cell gene mutation and chromosomal aberration test (10µg/ml of culture medium) and micronucleus test (500mg per kg. b.wt in 0.5% Caboxy methyl cellulose), for evaluation of its antimutagenicity/adaptive response.

Bacterial reverse mutation (Ames) test

The *Salmonella typhimurium* bacterial strains TA 1537, TA 98, TA 100 and TA 102 were obtained from Molecular Toxicology. INC, Boone. NC 28607, USA and used. Bacterial culture disc was added to 10ml oxoid nutrient broth and incubated for 15 hours at 37°C. After incubation bacterial cell count was adjusted to 1-2 x 10^9/ml with oxoid nutrient broth and used for the assay. Study was conducted both in the presence and absence of metabolic activation system.S9 mix (10%v/v) was used for the treatment in the presence and phosphate buffer saline was used in the absence of metabolic activation system.

Ames test was performed by following pre-incubation method [15, 17] for the evaluating antimutagenicity of quercetin. 100µl of quercetin (0.5mg in DMSO), 100µl of *Salmonella thphimurium* test strain (1-2 x 10^9/ml) and 100µl of positive mutagen and 0.5ml of phosphate buffer saline (PBS) or S9 mix (10%v/v) was added to glass tube and incubated for 20-30 min. at 37°C in water bath. After incubation culture mixture was added to 2 ml top agar tube and poured on Minimal Glucose Agar (MGA) plates. Triplicate plates were used for each concentration. Plates were incubated for 48 hours at 37°C and colonies were counted manually.

Positive mutagen 2-aminoanthracene, benzo (a) pyrene, bendamustine hydrochloride, cyclophosphamide monohydrate, hydralazine and etoposide were applied in the presence and 9-aminoacridine, sodium azide, 2-nitrofluorene, hydralazine, etoposide, methyl methane sulphonate were applied in the absence of metabolic activation system. However hydralazine and etoposide were applied both in the presence and absence of metabolic activation system. Based on mutagenic response demonstrated in author laboratory specific test stains were selected for each mutagen.

Table 2: Quercetin antimutagenicity in Ames test using *salmonella typhimurium* strains

Strain	Absence of metabolic activation (-S9)				Presence of metabolic activation (+S9)			
	NC	Mutagen	Quercetin +Mutagen	% IM	NC	Mutagen	Quercetin +Mutagen	%IM
TA 1537	8	208	324	Nd	8	Nt	Nt	Nt
TA 98	23	662	552	16.62	27	884	116	86.88
TA 100	108	1748	882	49.55*	125	1020	1203	Nd€
	108	766	413	46.08^		500	136	72.70£
TA 102	296	980	579	40.92#	311	812	826	Nd¥
		900	984	Nd$		900	1115	Nd^
		862	708	17.87^		1178	894	24.11$

NC-Negative control, %IM-inhibition of mutagenicity, Nd-Not detected, Nt-Not tested.

Mutagen (-S9): TA 1537-9 Amino acridine (50µg/plate), TA 98-2Nitro fluorine (7.5µg/plate), TA 100-*Sodium azide (5µg/plate), TA 102-#Methyl methane sulphonate (10µg/plate), $Etoposide(5mg/plate), ^Hydralazine(625µg/plate).

Mutagen (+S9): TA 98-Benzo (a)pyrene (5µg/plate), TA 100-€2Amino anthracene (10µg/plate), £Cyclophosphamide monohydrate (10µg/plate), TA 102-¥Bendamustine hydrochloride (5mg/plate), $Etoposide (5mg/plate). *%IM calculation: 1-[Colonies in quercetin+mutagen plate/colonies in mutagen plate] x 100.* [7].

Chromosomal aberration test (CAT)

CHO-K1 cells (ATCC No. CCL-61) were used from Palamur Biosciences Pvt. Ltd., cell line repository. Cells were cultured in Hams F12 K medium supplemented with 10% fetal bovine serum, penicillin (100IU/ml), amphotericin B (0.25µg/ml) and streptomycin (100µg/ml) and incubated in a CO_2 incubator at 37ºC and 5% CO_2. Culture was maintained by sub-culturing with 2-3x10^5cells/ml per each passage in a 75cm^2 culture flasks (Nunc 156367).

Monolayers of culture (2-3 x 10^5/ml) were exposed to 10µg/ml of quercetin. After 12 hours (equal to one generation time) incubation 10µl of reference mutagens were added to the culture medium. Benzo(a)pyrene (5µg/ml) and cyclophosphamide monohydrate (25µg/ml) was tested in the presence (+S9) and methyl methane sulphonate (20µg/ml) in the absence of metabolic activation system. Cells were incubated for further 4 hours, after incubation cells were washed with Dulbecco's phosphate buffer saline and fresh culture medium was added. Cells were further incubated for 1.5 normal cell cycle lengths (18hours) from the beginning of treatment. At 16th hour, 0.4µg colchicine (metaphase arresting agent) was added to each 1ml of culture. After 18hours incubation, cells were collected by trypsinisation and subjected to hypotonic treatment (8ml of 0.075M KCL solution). During hypotonic treatment tubes were incubated in a water bath at 37ºC for 25 min; centrifuged and the pellet was fixed in Carnoy's fixative (3:1 methanol: acetic acid). The cell pellet was prepared again and resuspended in a small volume of fixative. The cell suspension was dropped on to cleaned chilled slides and stained with 5% giemsa stain and mounted with DPX mount. Four slides were prepared from each concentration. 300 well spread metaphase cells from each concentration (include replicate 1 & 2) were observed for structural chromosomal aberrations under 100x objective of microscope. All the slides were coded prior to the screening [18, 19] and decoded after slide scoring.

Table 3: Adaptive response and antimutagenicity in chromosomal aberration test (CAT)

Dose (µg/ml)	%MI	% Aberrant cells	Reduction in % Aberrant cells
Absence of metabolic activation system (-S9)			
Negative control (0.0)	5.4	1.00	NA
Quercetin (10)	5.0	1.67	NA
Methyl methane sulphonate (20)	3.1	14.0	NA
Quercetin+ Methyl methane sulphonate (10+20)	3.6	10.33	28.2
Presence of metabolic activation system (+S9)			
Dose (µg/ml)	%MI	% Aberrant cells	Reduction in % Aberrant cells
Negative control (0.0)	5.8	1.33	NA
Quercetin (10)	5.2	2.00	NA
Benzo(a)pyrene (5)	2.8	16.00	NA
Cyclophosphamide monohydrate (25)	3.6	18.67	NA
Quercetin+ Benzo(a)pyrene (10+5)	3.4	10.00	40.9
Quercetin+ Cyclophosphamide monohydrate (10+25)	3.6	12.33	36.7

Mitotic index was calculated by formula: No. of metaphase cells/Total number of cells counted (1000 cells) x 100. %Reduction calculation: N. Aberrant cells by the mutagen – N. Aberrant cells by quercetin+mutagen / N. Aberrant cells by the mutagen- N. Aberrant cells in negative control*100., [19]. N. - number of, %MI-mitotic index, NA-Not applicable.

Cell gene mutation test (CGM) using L5178Y TK+/- mouse lymphoma cells

L5178Y TK+/- cells (ATCC No. CRL-9518) were used from Palamur Biosciences Pvt. Ltd., cell line repository. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100IU/ml), amphotericin B (0.25µg/ml) and streptomycin (100µg/ml) and incubated in a CO_2 incubator at 37ºC and 5% CO_2. Culture was maintained by sub-culturing with 2-3x10^5cells/ml per each passage in a 25cm^2 culture flasks.

Suspensions of cells (5-6x10^5/ml) were treated with 10µg/ml of quercetin. After 13 hours incubation 10µl of reference mutagens were added to the culture medium. Benzo(a)pyrene (5µg/ml), bendamustine hydrochloride (20µg/ml) and cyclophosphamide monohydrate (20µg/ml) was tested in the presence (+S9) and methyl methane sulphonate (20µg/ml) and etoposide (0.2µg/ml) in the absence of metabolic activation system. Cells were incubated

for further 4 hours and the treatment medium was replaced with fresh culture medium by centrifugation (1200 rpm for 10 min.). After treatment cells were allowed for mutation expression for 2 days and plated for cloning efficiency and trifluorothymidine (TFT) resistance. During expression, cells were sub-cultured to maintain 5-6 x 10^5cells/ml. Cell count was adjusted to 8 cells/ml of complete medium for viability plating and 10000 cells/ml of complete medium with TFT (3μg/ml) for TFT resistance colonies to calculate mutation frequency. The cells (0.2ml/well) were added to 96 well plates and incubated for 12 days in a CO_2 incubator and colonies were counted under inverted microscope (Olympus CKX41SF) [20].

Table 4: Adaptive response and antimutagenicity in cell gene mutation test (CGM)

Absence of metabolic activation system (-S9)			
Dose (μg/ml)	%Cloning efficieny (CE)	Mutation frequency (MF)	%Reduction in MF
Negative control (0.0)	81.65	54.99	NA
Quercetin (10)	53.94	88.62	NA
Methyl methane sulphonate (20)	30.96	393.41	NA
Etoposide (0.2)	22.02	289.74	NA
Quercetin+ Methyl methane sulphonate (10+20)	35.39	257.70	40.1
Quercetin+ Etoposide (10+0.2)	24.37	238.00	22.0
Presence of metabolic activation system (+S9)			
Dose (μg/ml)	%Cloning efficieny (CE)	Mutation frequency (MF)	%Reduction in MF
Negative control (0.0)	85.36	73.10	NA
Quercetin (10)	64.87	84.78	NA
Benzo(a)pyrene (5)	33.69	584.45	NA
Cyclophosphamide monohydrate (25)	35.96	515.85	NA
Bendamustine hydrochloride (20)	34.25	476.79	NA
Quercetin+Benzo(a)pyrene (10+5)	38.92	347.64	46.3
Quercetin+ Cyclophosphamide monohydrate (10+25)	40.16	319.97	44.2
Quercetin+ Bendamustine hydrochloride (10+20)	43.98	487.95	Nd
%cloning efficiency: -ln P(0)/1.6x100, Mutation frequency = (PE mutant/PE viable) x 10^6., [16]. %Reduction in MF calculation: MF by the mutagen – MF by the mutagen+quercetin / MF by the mutagen- MF in negative control*100. MF-mutation frequency, NA-Not applicable, Nd-Not detected.			

Mice bone marrow micronucleus test

The experiments were performed on Swiss albino mice (8 weeks old) with weight variation ± 20% of the mean weight of each sex. All experimental procedures were approved by the Institutional Animal Ethics Committee. The mice were housed in separate polypropylene cages in experimental room. All the animals were maintained at 22 ± 2°C and the relative humidity set at 40-70% with a 12:12-hour's light-dark cycle. They were fed with standard pellet feed and UV sterilized water *ad libitum*. Mice were acclimatized in the experimental room for five days before beginning the experiment.

Grouping and treatment

Swiss albino mice were divided into six groups viz., G1 (Vehicle control), G2 (Adaptive dose; quercetin), G3 (challenge dose 1: benzo(a)pyrene), G4 (challenge dose 2: cyclophosphamide monohydrate), G5 (quercetin+benzo(a)pyrene) and G6 (quercetin+cyclophosphamide monohydrate). Each group comprises of ten animals (5M+5F: females were nulliparous and

non-pregnant). G1 mice were treated with 0.5% carboxy methyl cellulose. G2 animals were treated with 500mg quercetin (oral), G3 animals with 200mg benzo(a)pyrene (intraperitoneal) and G4 animals with 40mg cyclophosphamide (oral) per kg b.wt. Group 5 and 6 animals were pre-treated with 500mg quercetin for 3hours before the administration of benzo(a)pyrene and cyclophosphamide. Doses volume was 1.0ml per 100g body weight at once for all groups. All the animals were sacrificed by CO_2 asphyxiation at 24hours following treatment. Bone marrow was collected and slides were prepared, stained and observed under microscope for micro nucleated polychromatic erythrocytes (MNPCE). A minimum of 2000 PCE were screened per each animal and the % micro nucleated polychromatic erythrocytes (%MNPCE) was calculated [21, 22].

Table 5: Adaptive response and antimutagenicity in mice bone marrow micronucleus test

Group	Sex	Mean MN PCE	Total PCE counted for MN	% MN PCE
Group 1 (vehicle control)	Male	5.0	10037	0.25±0.05
	Female	4.8	10037	0.24±0.02
Group 2 (quercetin 500mg/kg b.wt.)	Male	7.2	10046	0.36±0.05
	Female	6.6	10045	0.33±0.06
Group 3 (BP 200mg/kg b.wt.)	Male	83.4	10037	4.15±0.38
	Female	84.6	10052	4.21±0.38
Group 4 (CP 40mg/kg b.wt.)	Male	71.8	10064	3.57±0.24
	Female	68.4	10038	3.41±0.52
Group 5 (Q+BP 500+200mg/kg b.wt.)	Male	54.0	10031	2.69±0.19
	Female	51.6	10076	2.56±0.29
Group 6 (Q+CP 500+40mg/kg b.wt.)	Male	40.0	10114	1.98±0.16
	Female	42.6	10048	2.12±0.19

Statistical analysis

Graph pad Prism 6.0 software was used for statistical analysis. Fisher's exact test was used to check the statistical significant (p value <0.05) difference between mutagen and in combination treatment groups in chromosomal aberration test. In Mice bone marrow micronucleus test, unpaired t-test was followed to check the statistical significant (p value <0.05) difference between mutagen and in combination treatment groups (quercetin+mutagen).

Results

Results of *in vitro* studies are the average of two individual experiments.

Bacterial reverse mutation (Ames) test

Ames test was performed according to the procedure detailed in methods; the colonies were counted manually and % inhibition of mutagenicity (IM) was calculated by formula detailed in table 2. The varied response of quercetin was observed against different types of mutagens used in the test. Quercetin did not show any antimutagenic activity against 9 aminoacridine, 2 amino anthracene, bendamustine hydrochloride and modest effect against 2 nitrofluorine, hydralazine and etoposide; whereas a significant inhibition of mutagenicity (%IM) due to quercetin antimutagenicity was observed against sodium azide, methyl methane sulphonate, benzo(a)pyrene and cyclophosphamide monohydrate. Significant inhibition of mutagenicity (%IM) was observed against hydralazine in the absence of metabolic activation system and the same effect was not observed in the presence of metabolic activation system.

Chromosomal Aberration Test (CAT)

Results of CAT were shown in table 3. Initially 1000 cells per each slide were counted for metaphase cells to calculate mitotic index because mitotic index is the major constrain for calculating cytotoxicity. A significant reduction in percent mitotic index was observed with selected mutagens both in the presence and absence of metabolic activation system when compared with negative control. However cytotoxicity due to quercetin treatment was not observed, while a slight reduction in cytotoxicity was

observed in combination of mutagen+quercetin when compared with mutagen alone, though it is statistically insignificant.

For each dose group 300 well spread metaphases with equally divided in two replicates with 20±2 chromosomes were observed for structural chromosomal aberrations. Mean % aberrant cells (excluding gaps) were considered for results evaluation. A significant reduction in percent aberrant cells was observed in selective combination of mutagen with quercetin, though the rate of quercetin induced reduction rate was little high in the presence of metabolic activation system (with benzo(a)pyrene and cyclophosphamide monohydrate) than in the absence (methyl methane sulphonate). However there was no significant difference in percent aberrant cells of quercetin when compared with negative control.

Cell gene mutation test (CGM)

After 12 days of incubation 96 well plates were observed for colonies (cloning efficiency/viability and TFT resistance) under inverted microscope and unaided eye manually, reported in table 4. Both small and large colonies were considered for evaluation of results. Cloning efficiency was calculated to determine the cytotoxicity of selected test compounds by comparing with negative control. While in TFT plates an increase in the number of small colony was observed because of clastogenic damage induced by the test mutagens.

A decrease in mutation frequency (%RMF) was observed in quercetin+mutagen combination when compared with mutagen alone, both in the presence (+S9) and absence (-S9) of metabolic activation system, though the effect is varied. The combination of quercetin+etoposide (in -S9) exhibited only slight %RMF, whereas %RMF was not observed with bendamustine hydrochloride (in+S9). Significant difference/decrease in %RMF was observed with remaining combinations (Q+MMS, Q+BP, Q+CP). The varied results strongly indicate that the adaptive response of quercetin is selective towards certain alkylating agents when tested in eukaryotic test systems *in vitro*. However quercetin treatment alone did not result significant increase in mutation frequency when compared to negative control.

Mice bone marrow micronucleus test

The incidence of micro nucleated polychromatic erythrocytes (MNPCE) was counted in 2000 PCE from the bone marrow sample of each animal (detailed in table 5). A significant increase in %MNPCE was observed in animals of both sex treated with mutagen alone (G3, G4), while there was no significant difference in %MNPCE was observed in negative control (G1) and quercetin treated animals (G2). However a significant reduction in %MNPCE was observed in animals treated with a combination of quercetin and mutagens such as; benzo(a)pyrene (G5) and cyclophosphamide monohydrate (G6).

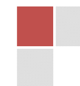
Discussion

Genetic toxicology tests are usually employed for the screening of new chemical entities and natural compounds in preclinical industry for the safety assessment and for the identification of antimutagenic and anticarcinogenic potential, as well as for revealing their mode of action. *In vitro* assays with prokaryotic and eukaryotic test systems are performed in combination with mammalian enzymes, this can provide the information about the metabolic activation system or detoxification that an agent may undergo *in vivo*. Hence we emphasise that the combination of different test systems with range of mutagens applied herein are important to gain explicit knowledge on the adaptive response and antimutagenicity of quercetin.

Quercetin mutagenicity was extensively reported in various *in vitro* mutagenicity assays and the same results were observed in the author's laboratory, long-term *in vivo* toxicity and carcinogenicity studies are failed to show the existence such correlation. Structure of the flavonoid ring; quercetin, kaempferol is responsible for their mutagen city [23]. Quercetin can bind directly to DNA and this contact may be one of the mechanism of *in vitro* mutagenicity and also strong mutagen in bacteria [24,25]. Quercetin is a pro-oxidant can oxidise nuclear envelope lipids and cause DNA strand breaks [26]. Based on these study results non-cytotoxic and non-mutagenic concentrations of quercetin was applied in the antimutagenicity and adaptive response assays. Mutagens that trail different mutagenic/DNA damaging pathways including; oxidation and direct DNA adduct or cross linking, alkylation, free radicals formation were selected to demonstrate the quercetin mode of action.

Quercetin shown considerable antimutagenicity against alkylating agent (CP, MMS), pro-mutagen and DNA adduct (benzo(a)pyrene) and direct damaging agent (sodium azide) in Ames test. Similar results were observed in cell gene mutation test using 5178Y TK+/- cells (CP, BP); make clear that quercetin protective role is closely similar in both prokaryotic and eukaryotic test systems against the same mutagens.

In chromosomal aberration test, significant reduction in benzo(a)pyrene (p value:0.03) and cyclophosphamide (p value:0.04) induced mutagenicity was observed in the presence of metabolic activation system where as the moderate effect was observed against methyl methane sulphonate induced mutagenicity in the absence of metabolic activation system. Defence against oxidative damage is the extensively reported attributes of polyphenols and may be the reason for using as dietary supplements. CYPA1 activity is especially needed in the mutagenicity/metabolism of pro-mutagens like 2 amino anthracene and benzo(a)pyrene. Concentration dependent inhibition of rat microsomal CYP1A1 activity by quercetin was well reported [27] and this is one of the mechanisms behind benzo(a)pyrene reduced



mutagenicity. Quercetin is an excellent free-radical scavenging antioxidant, protects against lipid peroxidation, chelates metal ions and forms inert complexes to prevent formation of hydroxyl radicals. These biological reactions strongly corroborate for its antimutagenic activity against free radical producing alkylating agent's benzo(a)prene and cyclophosphamide monohydrate.

Quercetin showed a clear antimutagensis effect against the direct DNA damaging agents; sodium azide and methyl methane sulphonate, shown protective role against sodium azide induced point mutations in *Salmonella* strain TA 100 and methyl methane sulphonate induced point mutations in thymidine kinase locus of L5178Y TK+/- cells, transitions/transversion mutations in TA 102 strain that detects DNA oxidative damage and clastogenicity in both *in vitro* and *in vivo* assays. This mechanism of protection may be due to the DNA repair genes expression, trap mutagenic compounds by direct binding and prevent DNA damage. Alba et al., confirmed the presence of the DNA-repair ogt-gene is essential for the antimutagenic action of *Rhoeo dischol*or plant extracts signifying that these extracts may progress the O^6 alkylguanine DNA alkyltransferase DNA repair enzymes activity. Furthermore mezougg et al., reported the extracts of different plants flavonoids could be acting as desmutagens; preventing DNA damage by direct binding to alkyl radicals [28, 29]. One more mechanism behind the protective role against these direct acting mutagens is the free radical scavenging and greatest antioxidant activity of quercetin.

Considering *in vitro* study results, quercetin protective role via adaptive response was tested in bone marrow chromosomal aberration in mice against BP and CP induced mutagenicity. Significant reduction in %micro nucleated polychromatic erythrocytes was observed in combination of quercetin and mutagen in either sex of animals. The observed effect was (p value: 0.001) closely similar to both tested mutagens indicates quercetin may decrease the catalysis of nitrosoylation of DNA alkylating agents BP and CP. Like many plant flavonoids quercetin may upregulate the glutathione S-transferase (GST) enzyme activity enhance the excretion of oxidising species or induce metallothionein an antioxidant enzymes. Quercetin may also inhibit the uptake of mutagens like benzo(a)pyrene [30] and also significantly prevent the formation of DNA adduct which was demonstrated in PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine)-DNA adduct formation studies in rat colon with many of plant polyphenols including quercetin [31].

Quercetin may induce cytotoxicity in cancer cells through multiple routes of action. It is plausible that quercetin interacts with DNA directly (DNA intercalation), and could be one of the mechanisms for inducing programmed cell death in both, cancer cell lines and tumor tissues by inducing the intrinsic apoptotic pathways. It is also shown to induce cell cycle arrest in S phase in various cancer cell lines. The several data consistently support the ability of quercetin as an effective anticancer agent because of low toxicity to normal tissue even at the concentrations that are highly detrimental to the cancer cells. The precise nature of quercetin to exhibit distinct biological effects on cancer and normal cells is the most promising feature of a chemical compound in the field of anticancer research.

Conclusion

Results of the current study indicate that quercetin demonstrated strong protective role against range of mutagens via majority of antimutagenicity and adaptive response mechanisms in a combination of *in vitro* and *in vivo* test systems, which could be the rationale for its potent anticancer activity. It is also evident from the results that the distinct antimutagenic activity of quercetin against mutagens of varied type may partly explain the suitability of quercetin as an anticancer agent against particular cancer types.

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Conflict(S) of Interest

Authors declare no conflict of interest.

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