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Observation of Counter Genotoxic Potential of Apigenin in the Bone Marrow Cell of Albino Mice

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ABSTRACT

Apigenin is regarded as one of the major flavonoids because of its presence and abundance in a variety of natural sources, including fruits and vegetables. It was found that a diet rich in flavones like Apigenin is related to a decreased risk of certain cancers, like cancers of the breast, digestive tract, skin, prostate and certain hematological malignancies. Numerous studies suggested that apigenin may be protective in other diseases that are affected by oxidative process such as cardiovascular and neurological disorders. In these studies, we used five optimum doses Apigenin suspension of 100, 200, 300, 400 and 500 µg/Kg body weights says AP_1, AP_2, AP_3, AP_4 and AP_5 respectively for this experiment. The methods adopted were total clastogeny and frequencies of aberrations in the bone marrow cells of albino mice *in vivo*. The data were collected at three durations of 16, 24, and 32 h for experimental studies. Apigenin significantly reduce number of aberrant cells ranges from 38.34, 50.38 and 46.15 percent for 16, 24 and 32 h respectively, frequency of aberrations per cell was also reduced significantly at $p<0.05$ level.

Key words: Genotoxicity, Clastogeny, Aberration, Aflatoxin B_1, Apigenin, *In vivo*

Apigenin is regarded as one of the major flavonoids because of its presence and abundance in a variety of natural sources, including fruits and vegetables. Major sources of apigenin include parsley, chamomile, celery, vinespinach, artichokes, and oregano. Among these, dried parsley is the richest source of apigenin, containing 45035 µg/g. Other sources of high apigenin content are chamomile (dried flower), celery seed, vinespinach, and Chinese celery, containing 3,000–5,000 µg/g, 786.5 µg/g, 622 µg/g, and 240.2 µg/g, respectively [1].

Assessment of the chemopreventive potential of apigenin in a colon carcinogenesis model induced by azoxymethane injection in rats demonstrated that dietary intake of this flavone (0.1%) triggered apoptosis of luminal surface colonocytes and reduced the incidence of aberrant crypt foci, the earliest identifiable lesions in the development and progression of colon cancer, particularly in the tumor initiation phase [2]. In the same model, subcutaneous injections of apigenin (0.75 and 1.5 mg/kg body weight) significantly decreased the incidence of peritoneal metastasis of intestinal adenocarcinomas [3]. Similarly, in mice, oral administration of apigenin reduced the number of polyp by the activation of p53, a tumor suppressor gene [4]. Overall, these results reflect the beneficial effect of apigenin against chemical- and mutation-induced carcinogenesis. Apigenin was shown to sensitize human colorectal carcinoma to apoptosis. A comparative study of nine dietary flavonoids was performed to examine their effect on cell growth in HCT116 human colon cancer cells [4]. Among the tested flavonoids, apigenin was found to be the most potent inhibitor of cell growth. Apigenin also induced apoptosis in other colon cancer cell lines, including HT-29, SW480, and LoVo [5]. The concentrations of apigenin used in these studies have typically been in the 1–90 µmol range and exposure times were 24 to 72 hours. Treatment of these cells with apigenin leads to the induction of the cell cycle inhibitor p21/WAF1, pro-apoptotic protein p53, and nonsteroidal anti-inflammatory drug-activated gene, caspase-3 activation, and DNA fragmentation. In present studies we also found Apigenin significantly reduced total clastogeny and also number of aberrations within aberrant cell.

MATERIALS AND METODS

The compound 4′,5,7-trihydroxyflavone is a natural flavone commonly referred to as apigenin. The name "apigenin", like many other flavonoids, is derived from *Apium* genus in Apiaceae (celery, carrot or parsley family, also known as Umbelliferae). The compound has hydroxyl groups at positions C-5 and C-7 of A-ring and C-4′ of B-ring and belongs to a class of flavonoids known as flavones. The molecular formula of apigenin is $C_{15}H_{10}O_5$ and molecular weight is MW 270.24; it is a yellow crystalline powder insoluble in water and soluble in dimethyl sulfoxide

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(DMSO). The experiments were performed with 8-10 week old Swiss Albino mice (25-35 g) following animal ethics of our institution, divided into 11 different group consisting of one mice in each group, 2 in control group (one normal control, one Aflatoxin induced) and 9 in test group *in vivo* with different concentration. All the mice were kept for overnight fasting. Aflatoxin B_1 and Apigenin in different concentrations were administered by subcutaneous injection into the plantar side of the left hind paw. We have selected five optimum doses of Apigenin suspension of 100, 200, 300, 400 & 500 µg/Kg. body weights for experiments noted as AP_1, AP_2, AP_3, AP_4 and AP_5 respectively in the Tables and literature. Mice were exposed to different test chemicals by appropriate routes and were sacrificed at sequential intervals at 16, 24, and 32 h of treatment. Mice have been treated with each test substance once at the selected doses. Samples were collected at three times after treatment. The central sampling interval was 8 h [6].

Immediately after sacrifice, the bone marrow was obtained, and exposed to hypotonic solution and fixed. The cells were spread on slides and stained. Chromosome preparations were made from bone marrow cells following above mentioned procedure. The metaphase cells were scored for chromosomal aberrations. Prior to sacrifice, mice were further treated with colchicines, a spindle inhibitor to arrest the cells in metaphase. The slides were stained in 10% aqueous Giemsa solution and 100 bone marrow metaphase cells from each animal were scored under code [6]. The types of chromosomal aberrations considered were chromatid and chromosome gaps, breaks, and fragments, exchanges and pulverization (severely damaged cells). The reduction factor due to Apigenin (AP) treatment was calculated using the formula:

$$\text{Percent reduction} = \frac{\text{Aberrant cells in Control} - \text{aberrant cells in } AFB_1 + AP}{\text{Aberrant cells in Control} - \text{aberrant cells in negative Control}}$$

RESULTS AND DISCUSSION

In this study we have conducted experiments using total number of chromosomal aberration per cell and percentage of clastogenic cell as biomarkers. Aflatoxin B_1 was taken as positive control It was found that due to Aflatoxin B1 at 16 h of treatment was 24.0 percent aberrant cell, but after the treatment with five different increasing concentrations of Apigenin along with Aflatoxin B_1, these values were reduced to 21.5, 18.7, 17.1, 16.0 and 14.8 percent respectively, whereas values for normal and DMSO control were 2.3 and 3.0 respectively. In terms of percent reduction in the number of aberrant cells, the observed values were 10.41, 22.08, 28.75, 33.34 and 38.34 percentage respectively for five different doses of Apigenin. The results show that Apigenin at each concentration can reduce the total aberrant cells significantly. This table also shows dose-dependent relationship between doses of Apigenin and the percent reduction of aberrant cells at 16 h (Table 1, Fig 1).

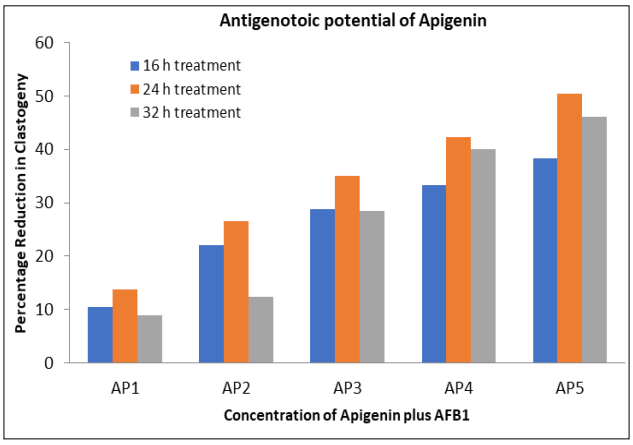

Fig 1 Showing *in vivo* anticlastogenic potential of Apigenin at 16h, 24 h and 32 h durations of treatment against AFB1 induced genotoxicity

Table 1 Effect of Apigenin (AP) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg.bw) at 16 h

Treatment	Apigenin (Y/kg.bw)	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		Percent reduction
			Gaps	Breaks	Fragments	Exchange	No.	(%)	
DH_2O	0	0	02	06	16	00	22	2.2	
DH_2O + DMSO	0	0	05	10	18	00	28	2.8	
AFB_1	0	19	88	96	112	16	240	24.0	
AP	AP_5	0	14	04	14	00	18	1.8	
AFB_1 + AP	AP_1	10	70	80	107	14	215	21.5	10.41
	AP_2	6	69	77	90	10	187	18.7	22.08
	AP_3	7	56	67	90	07	171	17.1	28.75
	AP_4	8	28	68	82	05	160	16.0	33.34
	AP_5	9	21	60	71	04	148	14.8	38.34

Similarly, at 16 h treatment duration the total frequencies of the aberrant cells were 433 for Aflatoxin B_1 alone which were reduced to 373, 308, 267, 240 and 210 for five different increasing doses of Apigenin respectively. These reduced values were statistically very significant. As the doses of Apigenin increases, the values of total frequency decreases, thus it shows dose-dependent inverse relationship. Cells with five or more than five aberrations were very few in numbers (Table 2).

After 24 h of treatments, the obtained value of percent aberrant cells was 24.00 percentage for Aflatoxin B_1 and 22.4, 19.1, 16.9, 15.0, and 12.9 percent for five different concentrations of Apigenin together with Aflatoxin B_1 respectively. The percentage reductions in the aberrant cells were 13.84, 26.53, 35.00, 42.30 and 50.38 percent, showed very significant anticlastogenic potential of different concentrations of Apigenin. It also shows inverse dose dependent relationship between the doses of Apigenin and percentage reduction of the aberrant cells (Table 3, Fig 1). When the frequencies of total number of aberrations in the aberrant cells were observed as presented in the (Table 4). It also shows similar trend as in the (Table 3) with increasing frequencies i.e., 480 for AFB1 and sequentially reduced to 403, 332, 277 and 221 aberrations in the aberrant cell noticed.

Table 2 Effect of Apigenin (AP) on the total numbers and types of frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg .bw) at 16 h

Treatment	Apigenin (Y/Kg.bw)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	978	20	02	00	0	0	0	24
DH_2O + DMSO	0	972	24	04	00	0	0	0	32
AFB_1	0	760	148	42	22	13	11	4	433
AP	AP_5	972	24	04	00	0	0	0	32
AFB_1 + AP	AP_1	785	136	39	19	10	8	3	373
	AP_2	813	125	31	16	7	6	2	308
	AP_3	829	119	27	13	7	4	1	267
	AP_4	840	115	24	11	6	4	0	240
	AP_5	852	111	21	9	5	2	0	210

Note: The animals were sacrificed 16 h after AFB_1 treatment. 1000 cells from 10 animals were analyzed for each point

Table 3 Effect of Apigenin (AP) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg.bw) at 24 h

Treatment	Apigenin (Y/kg.bw)	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		Percent reduction
			Gaps	Breaks	Fragments	Exchange	No.	(%)	
DH_2O	0	0	02	10	12	00	22	2.2	
DMSO	0	0	02	03	22	00	25	2.5	
AFB_1		36	204	100	130	15	260	26.0	
AP	AP_5	0	2	08	16	00	24	2.4	
AFB_1 + AP	AP_1	10	52	88	112	12	224	22.4	13.84
	AP_2	8	28	70	101	10	191	19.1	26.53
	AP_3	8	21	65	90	7	169	16.9	35.00
	AP_4	6	15	62	78	05	150	15.0	42.30
	AP_5	8	14	58	65	03	129	12.9	50.38

Table 4 Effect of Apigenin (AP) on the total numbers and types of frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg .bw) at 24 h

Treatment	Apigenin (Y/Kg.bw)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	979	18	03	00	00	00	00	24
DH_2O + DMSO	0	974	23	03	00	00	00	00	29
AFB_1	0	760	109	49	36	23	13	10	537
AP	AP_5	975	22	03	00	00	00	00	28
AFB_1 + AP	AP_1	776	108	44	33	20	12	07	480
	AP_2	809	92	40	27	17	10	05	403
	AP_3	831	88	37	21	13	07	03	332
	AP_4	850	84	33	17	09	04	03	277
	AP_5	871	79	29	13	05	03	00	221

Note: The animals were sacrificed 16 h after AFB_1 treatment. 1000 cells from 10 animals were analyzed for each point

When the experiment was conducted for 32 h the values obtained were 26.0 for Aflatoxin B_1 alone and 23.7, 22.8, 18.6, 15.6, 14.00 percent for 1st to 5th concentrations of Apigenin plus Aflatoxin B_1. When percentage reductions in clastogeny were calculated Apigenin showing that it significantly reduced the aberrant cells. The values for five different concentrations of Apigenin were 08.84, 12.30, 28.46, 40.00, and 46.15 percent respectively. (Table 5, Fig 1).

Table 5 Effect of Apigenin (AP) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg.bw) at 32 h

Treatment	Apigenin (Y/kg.bw)	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		Percent reduction
			Gaps	Breaks	Fragments	Exchange	No.	(%)	
DH_2O	0	0	03	05	16	00	21	2.1	
DMSO	0	0	03	02	24	00	26	2.6	
AFB_1	0	21	188	124	112	12	260	26.0	
AP	AP_5	0	03	05	16	00	21	2.1	
AFB_1 + AP	AP_1	15	160	115	102	10	237	23.7	08.84
	AP_2	12	131	97	105	08	228	22.8	12.30
	AP_3	8	120	88	88	05	186	18.6	28.46
	AP_4	6	90	79	75	01	156	15.6	40.00
	AP_5	8	62	76	65	00	140	14.0	46.15

Table 6 Effect of Apigenin (AP) on the total numbers and types of frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (AFB_1 x/kg .bw) at 32 h

Treatment	Apigenin (Y/Kg.bw)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	978	20	02	00	00	00	00	24
DH_2O + DMSO	0	975	22	03	00	00	00	00	28
AFB_1	0	740	102	86	29	20	14	09	567
AP	AP_5	976	22	02	00	00	00	00	26
AFB_1 + AP	AP_1	763	96	81	25	17	12	06	501
	AP_2	772	96	80	23	15	10	04	463
	AP_3	814	84	63	18	12	07	02	361
	AP_4	844	75	56	15	08	02	00	274
	AP_5	860	70	49	12	07	02	00	242

Note: The animals were sacrificed 32 h after AFB_1 treatment. 1000 cells from 10 animals were analyzed for each point

The total frequencies of aberrations in the aberrant cells were 567 for Aflatoxin B_1 and 501, 463, 361, 274 and 242 for 1st to 5th concentration of Apigenin along with Aflatoxin B_1. Thus, frequencies were also reduced significantly and show dose dependent relationship between the frequency and doses of Apigenin (Table 6).

The chemopreventive effect of apigenin was explored in many *in vivo* studies and tested doses, treatment frequencies and administration routes of apigenin. The oral administration of apigenin 50 µg/mice for 20 weeks reduced tumor volumes and induced complete abolishment of distant organ metastasis in the transgenic adenocarcinoma of a mouse prostate (TRAMP) model. This effect was attributed to the suppression of the phosphoinositide 3-kinase (PI3K)/Akt/box O-signaling pathway [7]. The same research group also showed that apigenin effectively suppressed prostate cancer progression by attenuation of insulin-like growth factor (IGF)-I/IGF binding protein-3 signaling and inhibition of angiogenesis and metastasis [8]. In an experiment oral administration of apigenin (2.5 mg/kg) in hamsters resulted in reduction of tumor volume and incidence, modulation of cell proliferation, apoptosis, inflammation, and angiogenesis markers, and modulation of phase I and II detoxification cascades in a 7,12-dimethyl benz[*a*]anthracene (DMBA)-induced experimental oral carcinogenesis model [9]. The chemoprotective effect of apigenin against oral carcinogenesis was further supported by studies reporting that this flavone lowered tumor incidence in DMBA-induced animal model [10]. The potential chemopreventive effects of apigenin on murine skin tumorigenesis initiated by DMBA and promoted by 12-O-tetradecanoylphorbol-13-acetate in SENCAR mice [11]. Their findings revealed that topical application of apigenin results in a marked reduction of incidence and number of papillomas as well as carcinomas.

An experiment conducted by Byun *et al*. [12] showed reduction of UVB-induced ear edema and inflammatory mediator COX-2 expression in the skin of SKH-1 hairless mouse, reflecting the potent chemopreventive activity of apigenin against UVB-induced skin inflammation. Another study showed that apigenin inhibited activation of the UVB-induced mammalian target of rapamycin (mTOR), cell proliferation, and cell cycle progression in mouse skin. The same study also demonstrated that apigenin inhibited UVB-induced mTOR signaling mainly through the activation of AMP-activated protein kinase (AMPK), rather than the suppression of Akt, even though UVB-induced mTOR activation is driven by PI3K/Akt signaling and apigenin is capable of blocking Akt phosphorylation/activation [13].

CONCLUSION

Here, we have discussed the beneficial roles of apigenin in the prevention and treatment of cancer through the reduction of genotoxic effects of environmental xenobiotics. Evidence from both *in vitro* and *in vivo* studies suggests that apigenin can trigger apoptosis or autophagy, which play pivotal roles in promotion and suppression of carcinogenesis. However, further in-depth investigations are needed to completely understand the mechanism of action and chemopreventive/therapeutic potential against human cancers. Apigenin has been found to be bioavailable following oral administration in rats and mice. The scientific evidence concerning apigenin is fascinating and deserves greater attention. As chemoprevention aims to stop the carcinogenic process or to prolong the onset of carcinogenesis by intervention with efficacious, non-toxic, and inexpensive agents to prevent, suppress, or reverse the malignant transformation, apigenin is one such agent that may satisfy most of these requirements. However, further information is required before apigenin can be brought to clinical trials. Overall, the findings reported in the literature suggest that apigenin offers great potential for further investigation and development as a cancer chemopreventive and therapeutic agent.

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