Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (1) January- March, pp. 177-184/Ahmad et al.
Research Article

EVALUATION OF ANTIGENOTOXIC POTENTIAL OF ELLAGIC ACID AGAINST AFLATOXIN B_1-INDUCED GENOTOXICITY

Md. Sultan Ahmad, Sheeba* and Rakesh Yadav
Department of Zoology, S.N (P.G) College, Azamgarh, U.P, 276001
Dept. of Zoology,D.S.College,Aligarh,U.P, 202002
**Author for Correspondence*

ABSTRACT
The antigenotoxic potential of Ellagic acid was demonstrated against the Aflatoxin B_1 induced genotoxicity. Studies were conducted on mice bone marrow cells using percentage of aberrant cell and frequency of total aberration as marker *in vivo.* Five optimum doses of Ellagic acid viz. 100, 200, 300, 400, and 450 mg /kg body weight *in vivo* and were studied, and found that Ellagic acid were significantly reduces the percentage of aberrant cells and frequencies of aberration In this experiments, we have notices that the antigenotoxic potential of Ellagic acid is depend on doses and duration of treatment.

Key Words*: Ellagic Acid, Chromosomal Aberration, Aberrant Cell, Aflatoxin B_1*

INTRODUCTION
Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* during growth and post harvest storage of a number of foodstuffs (POHLAND AND WOOD, 1991). The four major aflatoxins produced are B1, B2, G1, and G2; has been known human carcinogen (ANONYMUS), and DNA-damaging agents. It is known to oxidized by the mixed function oxygenases of the liver P-450 system; results in a reactive 8,9 epoxide production, which attack certain guanine residues in double stranded DNA (ANONYMUS).
We have earlier studied some natural products, like β-carotene, curcumin, genistein and vitamins of B-complex, C and E, which were antigenotoxic in human lymphocyte cultures (AHMAD *et al.*, 2002, 2004). In the present study, we have examined the antigenotoxic potential of ellagic acid and found that it significantly reduces the genotoxicity of AFB1.

Ellagic acid is a complex planar molecule, found abundantly in various fruits, nuts and vegetables, especially in freeze-dried raspberries. It is found in the form of ellagitannins; after hydrolysis it yielded ellagic acid. Ellagic acid is a very stable compound and is readily absorbed through the gastro-intestinal system. Ellagic acid is a powerful antioxidant that is able to bind to cancer-causing chemicals and neutralize them, thereby rendering them harmless (KRESTY *et al.*, 2001). It has been shown to inhibit the CYP1A1-dependent activation of [B (a) P], to bind to DNA, and reduce the formation of O^6-methyl guanine by methylating carcinogens; and to induce the phase-2 detoxification enzymes glutathion-S-transferase (BARCH *et al*, 1996)). Ellagic acid has been found to cause apoptosis in cancer cells in the laboratory (NARYANAN *et al*, 1999). In the present study, we have evaluated the antigenotoxic effects of ellagic acid in two test systems at three time durations against Aflatoxin B1-induced genotoxicity.

MATERIALS AND METHODS
The experiments were performed with 8-10 weeks old Albino mice (25-35 G) obtained from J.N. Medical College, Aligarh. Mice were exposed to different test chemicals by appropriate routes (I.P injection) and were sacrificed at sequential intervals at 16, 24, and 32 hours of treatment). Mice have been treated with the each test substance (as shown in the tables) once at the selected doses. Samples have been collected at three times after treatment. The central sampling interval was 24 h. Since cell cycle kinetics can be influenced by the test substances, one earlier and one later sampling interval adequately spaced within the range of 6 to 48 h, have been applied, where the additional dose levels were tested in a subsequent experiment, samples have been taken at the predetermined most sensitive intervals.

Immediately after sacrifice, the bone marrow have been obtained, exposed to hypotonic solution and fixed. The cells were spread on slides and stained. Chromosome preparations were made from bone marrow cells.

Table 1: List of chemicals, their concentrations and its sources

Chemicals	Doses *in vivo* (mg/kg.bw)	Source
Aflatoxin B1	500	Sigma, St. Louis, MO
Ellagic acid	100, 200, 300, 400, 450	Sigma, St. Louis, MO
DMSO	50	Sigma, St. Louis, MO

The cells were collected by centrifugation (10 min, 1200 rpm), hypotonic treatment (0.075 KCl) was given for 10-12 min at 37^0C and the recollected cells after centrifugation were fixed in methanol: acetic acid (3:1). DMSO and Aflatoxin B1 were used as negative and positive controls, respectively. Preparation of slides, staining and scanning was done under code. Aberrations were scored as per HUNDAL *et al* (1997).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. 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 Ellagic acid treatment was calculated using the formula:

$$\% \text{ Reduction} = \frac{\text{(Aberrant cells in control \_ aberrant cells in AFB1+Ellagic acid)}}{\text{(Aberrant cells in control \_ aberrant cells in negative control).}}$$

RESULTS AND DISCUSSION

After 16 h of treatments the percent aberrant cells were 6.7, 6.3, 6.6, 7.0, and 7.0 of five different concentrations of ellagic acid respectively against 7.2% of aberrant cells induced by Aflatoxin B_1. Fragments types of aberrations were most prominent followed by breaks and gaps. In terms of percentage reduction in the frequencies of aberrant cells the values were 9.25, 16.67, 11.12, 3.70 and 3.70 against five different concentrations of ellagic acid respectively. The maximum effect was 16.67% of the EL_2 concentration of ellagic acid (Table 2).

The gross effect on the total number of frequencies per thousand cells was 99, 82, 80, 88 and 92 of five different concentrations of ellagic acid against 100 of Aflatoxin B1 alone. The normal values were 22 for distilled water treatment and 24 and 25 for DMSO and ellagic acid alone treatment. It shows almost negligible effect on the total number of frequencies (Table 3). Most of the cells have one or two aberrations per cell. When the treatment durations were increases to 24 h, the effects were following similar trends, but with increasing values. The observed values were 11.1, 10.6, 11.2, 11.7 and 12.3 of five concentrations of ellagic acid respectively against 12.1 of Aflatoxin B_1 alone, whereas normal values were 1.7, 2.0 and 2.1 for pure water, DMSO and ellagic acid alone respectively. Only EL_2 concentration shows a noticeable effect on the percentage reductions of aberrant cell (Table 4).

Effect of Ellagic acid on the frequent of aberrations per cell and the total number of aberrations were also not statistically significant. The total aberrations per thousand cells were 188, 175, 177, 186 and 199 for Ellagic acid with Aflatoxin B_1 alone (Table 5).

During 32 h exposure, the aberrant cells observed were 14.9 percent for Aflatoxin B_1, whereas 13.9, 13.1, 11.9, 11.6 and 11.8 for five different doses of ellagic acid along with Aflatoxin B_1, against the normal values for control was 2.0 percent. The effects on reduction in aberrant cells were 7.75, 13.95, 23.25,

25.58 and 24.03 percent respectively, which were statistically significant. It also shows almost dose-dependent relationship, although the highest doses were not much effective in comparison to their preceding doses. More exchange types of aberrations were noticed in contrast to the previous two durations of treatment (Table 6 &7) .

Table 2: Effect of ellagic acid (EL) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (x/kg. bw), at 16 h of exposure.

Treatment	Ellagic acid (Y/kg.bw)	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		(%) Red.
			Gaps	Breaks	Fragments	Exchange	No.	(%)	
DH_2O	0	0	2	2	18	0	18	(1.8)	---
DH_2O + DMSO	0	0	2	3	19	0	21	(2..1)	---
AFB_1	0	17	30	15	51	2	72	(7..2)	---
Ellagic acid	EL_5	0	3	3	19	0	20	(2.0)	---
AFB_1 + Ellagic acid	EL_1	9	31	15	51	1	67	(6.7)	9.25
	EL_2	5	26	13	43	0	63	(6.3)	16.67*
	EL_3	5	24	12	44	0	66	(6.6)	11.12
	EL_4	7	29	13	46	0	70	(7.0)	3.70
	EL_5	9	30	14	48	0	70	(7.0)	9.70

Table 3: Effect of ellagic acid (EL) on the frequency of number of chromosome aberrations induced by Aflatoxin B_1 (x/kg), at 16 h of exposure.

Treatment	Ellagic acid (Y/kg.bw)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	982	16	2	0	0	0	0	20
DH_2O + DMSO	0	981	18	1	0	0	0	0	20
AFB_1	0	851	108	25	6	4	3	3	228
Ellagic acid	EL_5	979	17	2	0	0	0	0	21
AFB_1 + Ellagic acid	EL_1	867	99	21	4	3	4	2	199
	EL_2	878	94	18	3	3	2	2	176*
	EL_3	889	87	16	2	3	2	1	154*
	EL_4	879	90	18	3	5	3	2	185*
	EL_5	870	97	19	4	4	4	2	197

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

Table 4: Effect of ellagic acid (EL) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (x/kg), at 24 h of treatment durations.

Treatment	Ellagic acid Y/kg.bw	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells		(%) Red.
			Gaps	Exchange	Breaks	Fragments	No.	(%)	
DH_2O	0	0	1	1	20	0	17	(1.7)	---
DMSO	0	0	2	2	20	0	20	(2.0)	---
AFB_1+ DMSO	0	9	25	19	153	5	121	(12.1)	---
Ellagic acid	EL_5	0	2	2	20	0	21	(2.1)	---
AFB_1 + Ellagic acid	EL_1	8	22	14	142	5	111	(11.1)	9.61
	EL_2	5	21	13	133	4	106	(10.6)	14.42
	EL_3	5	20	15	136	3	112	(11.2)	8.65
	EL_4	7	21	14	145	3	117	(11.7)	3.84
	EL_5	7	22	17	152	4	123	(12.3)	---

Table 5: Effect of ellagic acid (EL) on the frequency of number of chromosome aberrations induced by Aflatoxin B_1 (x/kg), at 24 h of exposure.

Treatment	Ellagic acid(Y/Kg)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	981	12	5	0	0	0	0	22
DMSO	0	979	16	4	0	0	0	0	24
AFB_1 + DMSO	0	870	86	14	8	6	2	5	207
Ellagic acid	EL_5	977	18	3	0	0	0	0	24
AFB_1 + Ellagic acid	EL_1	874	80	13	7	4	2	5	188
	EL_2	877	78	11	6	4	3	4	175*
	EL_3	880	86	12	4	3	3	4	177
	EL_4	875	88	13	6	4	2	4	186
	EL_5	873	92	13	6	5	3	4	199

Note: The animals were killed 24 h after AFB_1 treatment. 1000 cells from 10 animals were analyzed for each point.

Table 6: Effect of ellagic acid (EL) on the frequency of cells with chromosome aberrations induced by Aflatoxin B_1 (x/kg), at 32 h of treatment durations.

Treatment	Ellagic acid Y/kg.bw	Cells with pulverized chromosome	Types of chromatid aberrations				Aberrant cells No. (%)	(%) Red.
			Gaps	Breaks	Fragments	Exchange		
DH_2O	0	0	6	3	13	0	20 (2.0)	---
DH_2O + DMSO	0	0	3	2	14	0	19 (1.9)	---
AFB_1+ DMSO	0	11	52	22	155	4	149 (14.9)	---
Ellagic acid	EL_5	0	5	2	15	0	22 (2..2)	---
AFB_1 + Ellagic acid	EL_1	8	47	20	145	4	139 (13.9)	7.75
	EL_2	5	44	17	136	3	131 (13.1)	13.95
	EL_3	5	38	14	112	3	119 (11.9)	23.25
	EL_4	4	35	12	114	2	116 (11.6)	25.58
	EL_5	6	41	15	111	2	118 (11.8)	24.03

Table 7: Effect of ellagic acid (EL) on the frequency of number of chromosome aberrations induced by Aflatoxin B_1 (x/kg), at 32 h of exposure.

Treatment	Ellagic acid(Y/kg)	Cells with aberrations							Total number of aberrations
		0	1	2	3	4	5	6-9	
DH_2O	0	980	18	2	0	0	0	0	22
DH_2O + DMSO	0	981	17	2	0	0	0	0	21
AFB_1 + DMSO	0	851	105	26	6	5	3	4	237
Ellagic acid	EL_5	988	18	2	0	0	0	0	22
AFB_1 + Ellagic acid	EL_1	861	98	24	6	4	4	3	220
	EL_2	869	95	22	5	3	3	3	203
	EL_3	881	89	21	4	2	1	2	170*
	EL_4	884	87	20	3	3	2	1	165*
	EL_5	882	87	21	3	4	2	1	171*

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

Results showed the role of ellagic acid that significantly reducing metaphase aberration, though the results of the anti-clastogenic effects were not very much promising; only higher duration of exposure of ellagic acid capable of reducing clastogeny significantly.

BOUKHARTA *et al* (1992), in their study showed that at a dose of 4 g/kg diet of Ellagic acid inhibited multiplicity of tumors induced by 4- (methyl nitrosamino) – 1 – (3- pyridyl)- 1- butanone in A/J mice by 54%. This inhibition was dose related between 0.06 and 4.0 g/kg diet. In the same work the bio distribution of ellagic acid was studied as a function of dose and time after gauges of ellagic acid. The levels of ellagic acid in the lung were directly proportional to the dose of ellagic acid between 0.2 and 2 mmol. The levels in liver tissues were 10-fold lower and reached a maximum 30 minutes after gavages. These results demonstrated that ellagic acid localizes preferentially in lung tissues and confirm that ellagic acid administered orally can inhibit lung tumorigenesis. THRESIAMMA *et al.,* (1998), studied on the induction of micronuclei and chromosomal aberrations produced by whole body exposure of γ-radiation (1.5-3.0 GY) in mice was found to be significantly inhibited by oral administration of ellagic acid (200 micro moles) per kilogram body weight, align with our study. SIGLIN *et al.,* (1995), observed the potential inhibitory effect of ellagic acid on the induction and progression of NMBA induced esophageal carcinogenesis in rats. Ellagic acid was administered only following NMBA treatment significantly reduced the incidence (66.7% versus 100% in NMBA controls) but not the multiplicity of esophageal tumors at the high dose (4000 ppm) level.

KHANDUJA *et al.,* (1999) have studied on the anticarcinogenic potential of ellagic acid and noticed that it significantly reduced tumor incidence to 20% from the control value of 72.2%. They observed that it suppressed the tumor incidence mainly by acting at the initiation phase of the carcinogenesis. Both ellagic acid and Quercetin caused a significant increase in GSH and decrease in NADPH and ascorbate dependent lipid peroxidation. In this study ellagic acid was found to be more effective in decreasing the lipid peroxidation and increasing the GSH. This may be one of the reasons for its observed better anticarcinogenic property as compared to quercetin. SONI *et al.,* (1997) studied on protective effect of food additives on aflatoxin-induced mutagenicity and hepato-carcinogenicity such as turmeric, asafetida etc including ellagic acid and found to inhibit the mutagenesis induced by AFB_1 in *Salmonella* tester strains TA 98 and TA 100. The results indicate the usefulness of antioxidants food additives in ameliorating aflatoxin induced mutagenicity and carcinogenicity. Ellagic acid was fed to trout fry following DMBA initiation in a tumor study. Incidence increased linearly with mean growth rate up to the 80g class, 69% incidence ellagic acid above this weight. In all growth classes, ellagic acid groups had lower incidence. Thus ellagic acid effectively suppressed tumors independently of growth inhibition (HARTTIG *et al.*, 1995).

Ellagic acid slows the growth of abnormal colon cells in humans prevents the development of cells infected with the human papilloma virus (HPV) linked to cervical cancer, and triggers apoptosis of prostate cancer cells. This apoptotic process may also have beneficial effects on breast lung, esophageal and skin cancer (NIXON, 1999, 2000).

Inhibition of carcinogenesis by Ellagic acid has been demonstrated in animals with esophagus, tongue, lung, colon, liver and skin tumors. Ellagic acid inhibits the initiation of tumors through a number of mechanisms including inhibition of metabolic activation of carcinogenic compounds (such as polycyclic hydrocarbons, nitroso containing chemicals or food preservatives and aflatoxins) into forms that induce cell DNA damage. Ellagic acid acts as a scavenger to "bind" cancer-causing chemicals making them inactive. It inhibits the ability of other chemicals to cause mutations in bacteria. In addition ellagic acid prevents binding of carcinogens to DNA, and reduces the incidence of cancer in cultured human cells exposed to carcinogens, ellagic acid inhibits mutagenesis and carcinogenesis by forming adducts with DNA, thus masking binding sites to be occupied by the mutagen or carcinogen (TEEL, 1986). The greatest inhibitory effect of ellagic acid and AFB_1 were incubated together with metabolic enzymes. Lower inhibition was apparent when the cells were first incubated with AFB_1 followed by a second incubation with ellagic acid alone and with metabolic enzymes. The result of these sequential incubation

studies indicates that one mechanism of inhibition could involve the formation of an AFB_1- ellagic acid chemical complex. The results indicate that AFB_1 is a direct-acting mutagen and that ellagic acid inhibits AFB_1 direct acting mutagenicity (LOARCA-PINA *et al.*, 1996).

Studies have found that ellagic acid forces cancerous cells to go through the normal apoptosis process without damaging healthy cells (ADAMS *et al.*, 2006). This is in contrast to many conventional cancer treatments, including chemotherapy, which cause the death of cancer cells and healthy cells indiscriminately, which can have a detrimental effect on the immune system. NARAYANAN *et al.*, (1999) studied the effect of ellagic acid on cell cycle events and apoptosis in cervical carcinoma cells. They found that ellagic acid induced apoptosis in cervical carcinoma cells after 72 h of treatment. Activation of the cdk or inhibitory protein P^{21} by ellagic acid suggests a role for ellagic acid in cell cycle regulation of cancer cells. It is a novel potent CK2 inhibitor. At present, ellagic acid represents the most potent known CK2 inhibitor (COZZA *et al.*, 2006). Our results on cell cycle kinetics also show similarity with the above discussion.

In this study we have found the protective effect of ellagic acid *in vivo* as Ellagic acid decreases the number of aberrant cells in bone marrow of albino mice and also decreases the aberrations per cell in the form of chromatid and chromosomes breaks exchanges and gaps etc. also strengthen the above discussions. We observed that ellagic acid is a very effective antimutagenic and anticarcinogenic indigenous natural chemopreventers.

REFERENCES

ADAMS LS, SEERAM NP, AGGARWAL BB, TAKADA Y, SAND D, HEBER D (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. 1: *Journal of Agriculture and Food Chemistry*. **54(3)** 980-5.

AHMAD MS, SHEEBA, AFZAL M (2004). Amelioration of genotoxic damage by certain phytoproducts in human lymphocyte cultures. *Chemico-Biological Interactions*; **149**: 107-115

AHMAD S, HODA A AND AFZAL M (2002). Additive action of vitamins of C and E against hydrocortisone-induced genotoxicity in human lymphocyte chromosomes. *Internation Journal of Vitamine and Nutritionaal Research*, **72(4)**: 204 -209.

BARCH DH, RUNDHANGEN LM, STONER GD, PILLAY NS, AND ROSCHE WA (1996). Structure-function relationships of the dietary anticarcinogen ellagic acid. *Carcinogenesis*. **17(2)**: 265-269.

BOUKHARTA M, JALBERT G AND CASTONGUAY A (1992). Biodistribution of ellagic acid and dose-related inhibition of lung tumorigenesis in A/J mice. *Nutrition and Cancer*. **18 (2)**: 181-189.

COZZA G, BONVINI P, ZORZI E. *et al* (2006) .Identification of ellagic acid as potent inhibitor of protein kinase CK2: a successful example of a virtual screening application. *Medical Chemistry*. **20; 49(8)**: 2363-6.

HARTTIG U, STONER G AND BAILEY G (1995). Tumour suppression and anti-initiation by dietary ellagic acid against 7, 12- dimethyl benz [a] anthracene in rainbow trout. *Proceeding of Annul. Meeting of American Association of Cancer Research.* **36**: A 739.

HUNDAL BS, DHILLON VS AND SIDHU IS (1997). Genotoxic potential of estrogens. *Mutation Reearchs*. **389**: 173 – 181.

IARC (1987). IARC monographs on the evaluation of carcinogenic risks to humans. Suppl. **7**, World Health Org; Intl. Agency for Research on cancer, Lyon, France

International Agency for Research on Cancer (IARC, 1993). 'Aflatoxins: Summaries and Evaluations.'. Volume. **56.**

KHANDUJA KL, GANDHI RK, PATHANIA V, AND SYAL N (1999). Prevention of N-nitrosodiethylamine-induced lung tumourigenesis by ellagic- acid and quercetin in mice. *Food and Chemical Toxicology*. **37 (4)**: 313 – 318.

KRESTY LA, MORSE MA, MORGAN C, CARLTON PS, LU J, GUPTA A, BLACKWOOD M AND STONER GD (2001). Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. *Cancer Research*. **61(16)**: 6112-6119

LOARCA-PINA G, KUZMICKY E, GONZALEZ DE ME FIA, KADO NY AND SIEH DH (1996). 'Antimutagenicity of ellagic acid against Aflatoxin B1 in the Salmonella micro suspension assay.' *Mutation Research*. **360(1)**: 15-21.

NARYANAN BA, GEOFFREY O, WILLINGHAM MC, RE GG AND NIXON DW (1999). $P^{53/21}$ (WAF_1 /ClP_1) expression and its possible role in G_1 arrest and apoptosis in ellagic acid treated cells. *Cancer Letter*.**136 (2)**: 215-221

NIXON DW (2000). Prostate cancer and nutrition .*JSC. Med. Assoc*., **96(2)**: 85-86.

NIXON DW (1999). Alternative and complementary therapies in oncology care. *Journal of .Clinical Oncology*.**17 (11)**: 35-37.

POHLAND AE AND WOOD GE (1991). Natural occurrence of mycotoxins. In. "Mycotoxins cancer and Health." Pennington center Nutritional series Vol. **1** (ed). G.A. Bray and D.H. Ryan. Pp. 32-52.

SIGLIN JC, BARCH DH AND STONER GD (1995). Effects of dietary phenethyl isothiocyanate, ellagic acid, salindac and calcium on the induction and progression on N-nitrosomethyl benzylamine – induced esophageal carcinogenesis in rats. *Carcinogenesis*; **16(5)**: 1101-1106.

SONI KB, LAHIRI M, CHACKRADEO P, BHIDE SV AND KUTTAN R (1997). Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. *Cancer Letter*. **115 (2)**: 129-133.

TEEL. R.W. (1986). Ellagic acid binding to DNA as a possible mechanism for its antimutagenic and anticarcinogenic action. *Cancer. Letter*. **30(3)**: 329-336.

THRESIAMMA KC, GEORGE J AND KUTTAN R.(1998). Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity. *Journal of Experimental and Clinical Cancer Research*. **17(4)** 431-434.