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Environmental mutagens and possibilities for prevention

Mutageni ambientali e possibili strategie per la prevenzione

Wagida A. Anwar
Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Summary

Humans are exposed to many physical or chemical genotoxic agents that can increase the probability of mutation. On the other hand, there are possible ways to prevent this effect. One of them is to prevent cellular injury by augmenting endogenous oxidative defences through the dietary intake of antioxidants such as vitamin C or vitamin E. Also olive oil has proved to have potent antioxidant properties. Several studies have been conducted during the last ten years to demonstrate the genotoxic effect of these environmental pollutants, and to investigate possible protective effects. In one of these studies, we investigated the mutagenic effects of two commonly used pyrethroid pesticides, cypermethrin and fenvalerateon, on bone marrow cells of adult male rats and the possible protective rôle played by olive oil and vitamin C. Results revealed that cypermethrin and fenvalerate induced a significant increase in the total number of chromosomal aberrations, and that more were observed with fenvalerate. Olive oil and vitamin C induced significant improvement of total aberrant cells at high doses (1/10 LD50). Aflatoxins contaminate many food products and are consequently of worldwide heath concern. Prevention of exposure to aflatoxins can be achieved either at

Riassunto

Gli uomini sono esposti a molti agenti genotossici fisici o chimici che possono aumentare la probabilità di mutazioni. D'altra parte, esistono possibili modi di impedire questo effetto. Uno di questi è prevenire il danno cellulare aumentando le difese ossidative endogene attraverso l'assunzione con la dieta di antiossidanti come la vitamina C o la E. È stato dimostrato che l'olio d'oliva ha potenti proprietà antiossidanti. Durante gli ultimi dieci anni, sono stati condotti numerosi studi per dimostrare gli effetti genotossici di questi inquinanti ambientali ed anche per investigare un possibile effetto protettivo. In uno di questi studi abbiamo indagato gli effetti mutageni di due pesticidi piretroidi comunemente usati, il cypermethrin e il fenvalerato, sulle cellule di midollo osseo di ratti adulti maschi e il possibile ruolo protettivo giocato dall'olio d'oliva e dalla vitamina C. I risultati hanno mostrato che il cypermethrin e il fenvalerato hanno indotto un significativo aumento nel numero totale di aberrazioni cromosomiche, maggiormente osservate con il fenvalerate. L'olio d'oliva e la vitamina C hanno prodotto un significativo miglioramento delle cellule aberranti totali alle alte dosi (1/10 LD50). Le aflatossine contaminano molti prodotti alimentari e sono di conseguenza un problema di salute a livello mondiale. La prevenzione dell'e-

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Address/Indirizzo: Dr. Wagida A. Anwar, Department of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University, Abassya, Cairo, Egypt - Tel./Fax +202/24837888 - E-mail: wanwar2@hotmail.com

community (via good agriculture practices) or individual levels (treatment or dietary interventions). Several trials were carried out to evaluate the effect of the processing steps of corn products on the destruction of aflatoxins in popcorn. The results of the trial indicated that the processing of popcorn had a significant effect on aflatoxin destruction. High temperature of preparation and treatment with 5% salt (sodium chloride) yielded the highest destruction rate. Several probiotic bacteria are able to bind aflatoxin B1 (AFB1) *in vitro*, including *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii subsp. shermanii JS*. A mixture of these two probiotics is used by the food and feed industry as biopreservative (Bioprofit), making it a promising candidate for future applications. A recent study was carried out to determine whether administration of probiotic bacteria could block the intestinal absorption of aflatoxin B1. Probiotic administration led to a statistically significant decrease in the urinary excretion of AFB-N7-guanine. Probiotic supplement reduces the biologically effective dose of aflatoxin exposure, and may thereby offer an effective dietary approach to prevent the development of liver cancer. An other example of these studies is that on the radioprotective rôle of vitamin C and E against gamma radiation-induced depletion in relative testicular weight and sperm shape abnormalities. Eur. J. Oncol., 13 (4), 219-227, 2008

Key words: **environment, pollution, mutagens, biomarkers, antimutagens, strategies for prevention**

sposizione alle aflatossine può essere ottenuta sia a livello di comunità (attraverso buone pratiche rurali) sia a livelli individuali (trattamento o intervento sulla dieta). Sono stati condotti molti studi per valutare l'effetto dei passaggi di processazione dei prodotti del grano sulla eliminazione delle aflatossine nel *popcorn*. I risultati di questi studi hanno rilevato che il processo di preparazione del *popcorn* ha un significativo ruolo sulla distruzione delle aflatossine. La temperatura di preparazione e trattamento con il sale al 5% (cloruro di sodio) ha fruttato la più alta velocità di rimozione. Molti batteri probiotici sono capaci di legare l'aflatossina B1 (AFB1) *in vitro*, inclusi il *Lactobacillus rhamnosus* LC-705 e il *Propionibacterium freudenreichii, sottospecie shermanii JS*. Una miscela di questi due probiotici è stata usata dall'industria del cibo e dell'alimentazione come bioconservante (Bioprofit), rendendola un promettente candidato per future applicazioni. Uno studio recente è stato condotto per determinare se l'ingestione di batteri probiotici possa bloccare l'assorbimento intestinale della aflatossina B1. L'assunzione del probiotico ha portato a una diminuzione statisticamente significativa della escrezione urinaria di AFB-N7-guanina. L'integratore probiotico riduce la dose effettiva biologica dell'esposizione alla aflatossina, e potrebbe oltretutto offrire un effettivo approccio alimentare per prevenire lo sviluppo del cancro epatico. Uno studio di intervento sull'uomo è attualmente in corso in Egitto su un gruppo di soggetti con provata esposizione di base alle aflatossine. Un altro esempio di questi studi è il ruolo radioprotettivo della vitamina C ed E verso la deplezione indotta dalle radiazioni gamma nel peso relativo del testicolo e le anomalie di forma dello sperma. Eur. J. Oncol., 13 (4), 219-227, 2008

Parole chiave: **ambiente, inquinamento, mutageni, biomarcatori, antimutageni, strategie di prevenzione**

Introduction

Humans are exposed to many physical (e.g., radiation) or chemical (e.g. pesticides and aflatoxins) genotoxic agents, that can increase the probability of mutation[1, 2].

The introduction and widespread use of pesticides continue in agricultural, commercial, recreational,

and home settings. As a result, these often very toxic substances pose a potential threat to people using them, especially if they are handled, mixed, or applied inappropriately or excessively[3]. Potential exposure from the environment can be estimated by environmental monitoring. Actual exposure (uptake) is measured by biological monitoring of human tissues and body fluids. Biomarkers are used to detect the presence of pesticides before adverse clinical health effects occur[3]. Agricultural workers exposed to pesticides showed increased incidence of chromosomal aberrations and sister chromatid exchanges[4]. Adverse effects may be caused not only by the active ingredients and the associated impurities, but also by the solvents, carriers, emulsifiers and other constituents of the formulated products[5]. Furthermore, children are at increased risk of pesticide poisoning because of their smaller size and because pesticides may be stored improperly or applied to surfaces that are more readily accessible by children[6]. The use of persistent organochlorine insecticides is being banded or restricted. However, residues of DDTs, lindane and other organochlorine insecticides were reported to persist in national water bodies and accumulated in fatty tissues of living organisms[7-9].

Occupational exposure pesticides may have a contributory rôle in the aetiology or progression of hepatocellular carcinoma (HCC). According to Ezzat *et al*[10], a major segment of the Egyptian population is employed in agriculture and uses pesticides routinely to control insects, weeds, rodents, and fungal infections of crops and livestock. A case-control study was carried out to investigate pesticides as environmental risk factors for HCC while taking into account viral risk factors: 236 subjects with confirmed HCC were recruited from the National Cancer Institute, Cairo University, Egypt, and 236 controls matched on sex, age group and urban-rural status recruited from the Orthopaedic Department of the same hospital. Among rural males, the adjusted odds ratio (OR) for organophosphorus compounds was 2.7 and for carbamates it was 2.9. No statistically significant associations between HCC and household application of pesticides were observed for urban males or for females. This study suggested that exposures to organophophorus and carbamate pesticides are additive risk factors to current HCV and HBV infection among rural males.

Exposure of rats to pyrethroids results in free radical-mediated liver damage as indicated by elevated hepatic peroxidation. Pyrethroids were found to produce significant oxidative stress in hepatic and cerebral tissues of rats as shown by the elevation of thiobarbituric acid reactive substances[11].

Pyrethrin has been used as an insecticide for several centuries. It is composed of esters of pyrethric and chrysanthemumic acid, which are obtained from the flower of *Chrysanthemum cinerifolium* and related species[12].

The widespread use of pyrethroid compounds in agriculture or public health stimulate the need to study their biochemical and haematological toxic effects. At the same time, attempts should be made to prevent or at least to reduce the toxicity of these compounds.

Aflatoxins

Aflatoxins contaminate many food products and are consequently of worldwide health concern. Aflatoxins are toxic and carcinogenic metabolites of moulds, mainly *Aspergillus flavus* and *parasiticum* that contaminate a variety of agricultural commodities, particularly peanuts, maize and cottonseed, in countries with hot and humid climates. Aflatoxin B1 (AFB1) is the major metabolite produced by these moulds. It has been shown that aflatoxins are potent carcinogens in a number of animal species. AFB1 has been classified by the International Agency of Research on Cancer as a group I carcinogen. It has been suspected as a causal agent in the induction of HCC[13]. While HBV and HCV may account for the majority of HCC in Egypt, there is suggestive evidence for an additional aetiologic rôle of aflatoxin in hepatocarcinogenesis. The rôle of aflatoxins in carcinogenesis is complicated by Hepatitis B virus infection[2, 14, 15].

Mohamed *et al*[16] detected a significantly higher percentage of aflatoxins in the serum of Egyptian patients with HCC compared to their controls, with a twofold increased risk. Aflatoxins may cause mutations in the tumour suppressor gene p53 that act as initiating agents, leading to liver cell hyperplasia and

HCC. In support of this hypothesis, El Kafrawy *et al*[17] documented the presence of p53 codon 249 mutations, associated with aflatoxin exposure, in a sample of HCC tumour tissues analyzed by gene chip analysis in Egypt.

Hifnawy *et al*[18] suggested that the progressive nature of HCV-related liver diseases was influenced by aflatoxin exposure. The quantitative identification of possible aflatoxin contamination in six urban and eleven rural areas in Egypt, using high performance liquid chromatography technique, revealed that the prevalence of AFB1 contamination in corn, wheat, peanut, lupine, white rice, cowpea, fava bean and brown rice was 64.7%, 53%, 53%, 47%, 47%, 41%, 29.4% and 29.4%, respectively. A positive correlation was found between aflatoxin and positive HCV-PCR together with liver disease progression to stage G3S3 that was indicative of HCC.

Several trials were carried out to evaluate the level of aflatoxins in food products in different governorates[19]. From Qaluobia and Kafr El-Sheikh Governorates, one hundred samples of imported and local wheat grains (*Triticum sativum*) were collected and examined for the natural occurrence of aflatoxins during 2000-2001. Results indicated that both local and imported samples were positive for AFB1 (17.5% and 20%, respectively), and the concentration ranged from 3-25 µg/kg. The level of aflatoxins was dependent on the area of collection as well as the season of the year.

During 2004, samples were collected from the Menofeya Governorate (4 different districts: Mnuf, Quisna, Shibin El-Kom and Tala) to survey the natural occurrence of aflatoxins in local and imported corn grains and some corn products. The results showed that the local and imported corn grains were naturally infected with fungi belonging to genera of *Aspergillus.* The concentrations of aflatoxin B1 (AFB1) ranged between 5.8 and 7.5 µg/kg corn grains, while the concentration of aflatoxin B2 (AFB2) varied from 2.7 to 4.1 µg/kg corn grains. On the other hand, the concentrations of aflatoxins G1 and G2 were 8.1 and 3.2 µg/kg corn grains, respectively. On the other hand, out of 15 samples of corn products (snacks, cornflakes and popcorn) collected from local markets, only one sample of cornflakes contained AFB1 and AFB2 at concentrations of 3.4 and 2.7 µg/kg cornflakes, respectively.

In a study to screen for biomarkers of aflatoxin exposure in Egypt, Polychronaki *et al*[20] assessed the level and frequency of breast milk AFM1 as a biomarker of maternal exposure. Breast milk samples were collected from a selected group of 388 Egyptian lactating mothers of children attending the New El-Qalyub Hospital, Qalyubiyah Governorate, during May-September 2003. Approximately 36% of mothers tested positive for AFM1. Non-working status, obesity, high corn oil consumption, number of children, and early lactation stage (<1 month), contributed to the occurrence of AF in breast milk.

The same research group continued their study[21], following up with fifty of those women who were AFM1-positive at baseline; they were revisited monthly for 12 months to assess the temporal variation in breast milk AFM1. In a multilevel regression model of the data there was a highly significant ($p<0.001$) effect of month of sampling on the frequency of AFM1 detection with summer months having the highest frequency (>80%) and winter months the lowest frequency (<20%) of detection. The duration of lactation and peanut consumption also contributed to the model. The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a knowledge driven basis for controlling the transfer of chemicals to infants.

Hassan *et al*[22] assessed the presence of aflatoxin (AFM1) in both mothers' milk and the infants' sera. Fifty healthy breast lactating mothers and their infants who were exclusively breast fed for at least 4 months were included. Twenty-four mothers (48%) and their infants had detectable levels of aflatoxin with the following mean contamination levels (ng/ml); mothers' serum of 8.9+/-4.2, mothers' milk of 1.9+/-0.6 and infants' serum of 1.8+/-0.9.

Prevention and control

Environmental health problems can be managed and prevented in different ways. There is a realization that governments should work hand in hand with non-governmental and international organizations to help improve human health. Research should be directed towards applied problem-solving within

the context of an evaluation of the practicability and scope of solution.

Since reactive oxygen metabolites (ROM) are generated during both normal and xenobiotic metabolism, they can be overproduced in several pathological conditions. Cells are naturally provided with protective enzymatic and non enzymatic antioxidants that counteract these potentially injurious oxidizing agents[23]. The accumulation of reactive oxygen metabolites in several cellular components is thought to be a major cause of molecular injury. One method to preclude adverse effects is to prevent cellular injury by augmenting endogenous oxidative defences through the dietary intake of antioxidants such as vitamin A, C or vitamin E[24].

Recently, attention has also focussed on a variety of non vitamin antioxidants such as phenolic compounds that might also contribute to cellular defense mechanisms[25]. These phenolic compounds are found in many plant species including olive oil, fruits and vegetables[26]. It has been postulated that the presence of olive oil in the Mediterranean diet can contribute to the lower incidence of coronary heart disease, prostate and colon cancers[27, 28].

Olive oil has potent antioxidant properties[29, 30]. Clinical studies supported the evidence on the physiological and anti-inflammatory effects of omega-3 fatty acids present in olive oil[31].

Youssif *et al*[32] investigated the mutagenic effects of two commonly used pyrethroid pesticides, cypermethrin and fenvalerate, on the bone marrow cells of adult male rats, and the possible protective rôle played by olive oil and vitamin C. Two dose levels were tried for each pesticide: 1/30 and 1/10 of LD50. Results (fig. 1) revealed that cypermethrin and fenvalerate induced a significant increase in the total number of chromosomal aberrations and that more were observed with fenvalerate. There was an increase in the frequency of both structural and numerical aberrations with both pyrethroids. The most frequent structural aberrations were chromatid deletions, gaps, centric fusion in cypermethrin toxicity, while fenvalerate caused deletions, acentric fragments, gaps and fragments in descending order. The numerical aberrations were represented by polyploidy. Both doses of pyrethroids induced genotoxic effects, but more were observed with the high doses. Olive oil and vitamin C induced significant improvement of total aberrant cells at high doses (1/10 LD50). Vitamin C improved the toxic effect of cypermethrin (1/30 LD50) to about normal control value.

In parallel, prevention of exposure to aflatoxins can be achieved either at community (via good agriculture practices) or individual levels (treatment or dietary interventions).

Amra *et al*[19] studied the effect of processing steps of corn products on the destruction of aflatoxins for popcorn. The results of the trial (fig. 2) indicated that the process of popcorn preparation had a significant effect on aflatoxin destruction. High temperature of preparation and treatment with 5% salt (sodium chloride) yielded the highest destruction rates (ranging from 90 to 97% depending on the specific compound measured). Such effects of processing of corn grains on the fate of residual aflatoxins should stimulate more trials aiming at the prevention of aflatoxin health hazards.

Several probiotic bacteria are able to bind AFB1 *in vitro*, including *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii subsp. shermanii* JS[34]. A mixture of these two probiotics is used by the food and feed industry as biopreservative (Bioprofit), making it a promising candidate for future applications. Consequently, ongoing studies in Egypt and elsewhere are investigating the *in vitro* and *ex vivo* ability of this probiotic mixture to bind AFB1. *In vitro* experiments, 57 to 66% of AFB1 was removed from the solution by the probiotic mixture, but only 38 to 47% could be extracted from the bacterial surface. In *ex vivo* experiments, only up to 25% of AFB1 was bound by bacteria, and tissue uptake of AFB1 was significantly reduced when probiotic bacteria were present in the duodenal loop. Furthermore, the effect of intestinal mucus on the bacterial binding ability was investigated *in vitro* and was found to significantly reduce AFB1 binding by the probiotic mixture. Further work needs to assess the detoxifying potential of probiotics in different experimental setups, and in different dietary interventions. For example, a recent study[35] was carried out among 90 healthy young males from Guangzhou, People's Republic of China, to determine whether the administration of probiotic bacteria could block the intestinal absorption of AFB1 and thereby lead to reduced urinary excretion

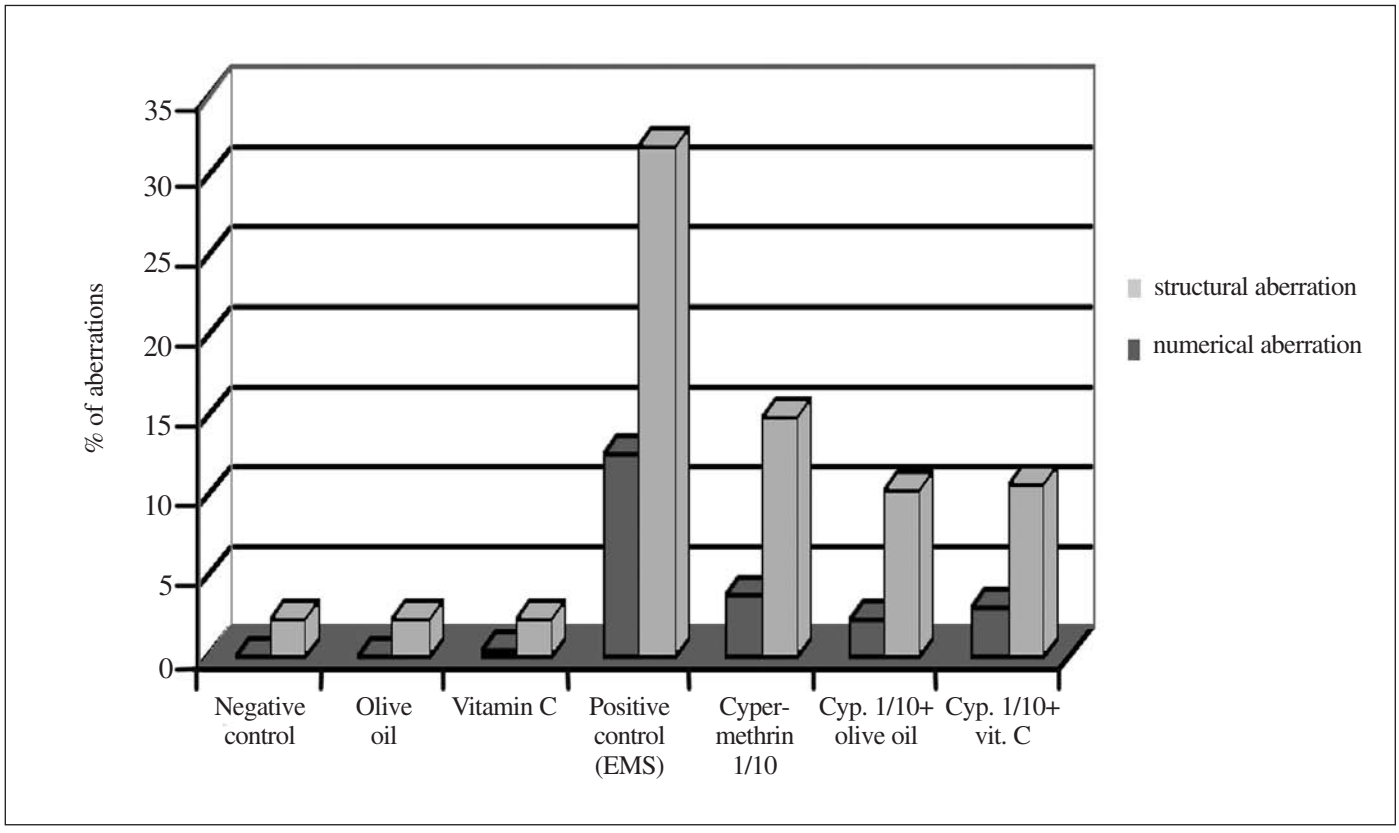

Fig. 1. Percentage of structural and numerical chromosomal aberrations in bone marrow cells due to cypermethrin 1/10 LD50 and olive oil or vitamin C treated rats
From Youssif *et al*[32]

This experimental work was carried out on eighty adult albino male Sprague Dawley rats of weight 80-100g. The clastogenic effect and the possible protective rôle played by olive oil and vitamin C were studied on bone marrow cells of adult male rats. Rats were divided into 5 main groups:

- negative control group
- positive control group ethyl methyl sulphonate
- pesticide treated group (Cypermethrin 1/10 LD50, Cypermethrin 1/30 LD50, Fenvalerate 1/10 LD50, Fenvalerate 1/30 LD50
- olive oil treated group (Olive oil, Olive oil + cyp. 1/10 LD50, Olive oil + cyp. 1/30 LD50, Olive oil + fen. 1/10 LD50, Olive oil + fen. 1/30 LD50)
- vitamin C treated group (Vitamin C, Vit. C + cyp. 1/10 LD50, Vit. C + cyp. 1/30 LD50, Vit. C + fen. 1/10 LD50, Vit. C + fen. 1/30 LD50

of aflatoxin B1-N7-guanine (AFB-N7-guanine), a marker for a biologically effective dose of aflatoxin exposure. Probiotic consumption led to a statistically significant decrease in the urinary excretion of AFB-N7-guanine (36% reduction at week 3 and 55% at week 5). Additionally, the likelihood for negative AFB-N7-guanine at weeks 3-5 was 2.89 times bigger in the probiotic arm compared with placebo.

Such probiotic supplementation reduces the biologically effective dose of aflatoxin exposure, and may thereby offer an effective dietary approach to prevent the development of liver cancer. To assess this possibility, an intervention study is ongoing now in Egypt in a group of human subjects proven to be exposed to aflatoxins at baseline.

The protective action of vitamins C and E against gamma radiation effects[36] was examined by studying the relative testicular weight and sperm shape abnormalities. The results of the study indicated that treatment with vitamin C, vitamin E or combination of both decreased the effect of irradiation but the average relative testicular weights of mice did not reach the control average weight except after 36 days of irradiation. No difference in sperm head abnormalities was found between the control group and non-irradiated animal groups treated with vitamin C

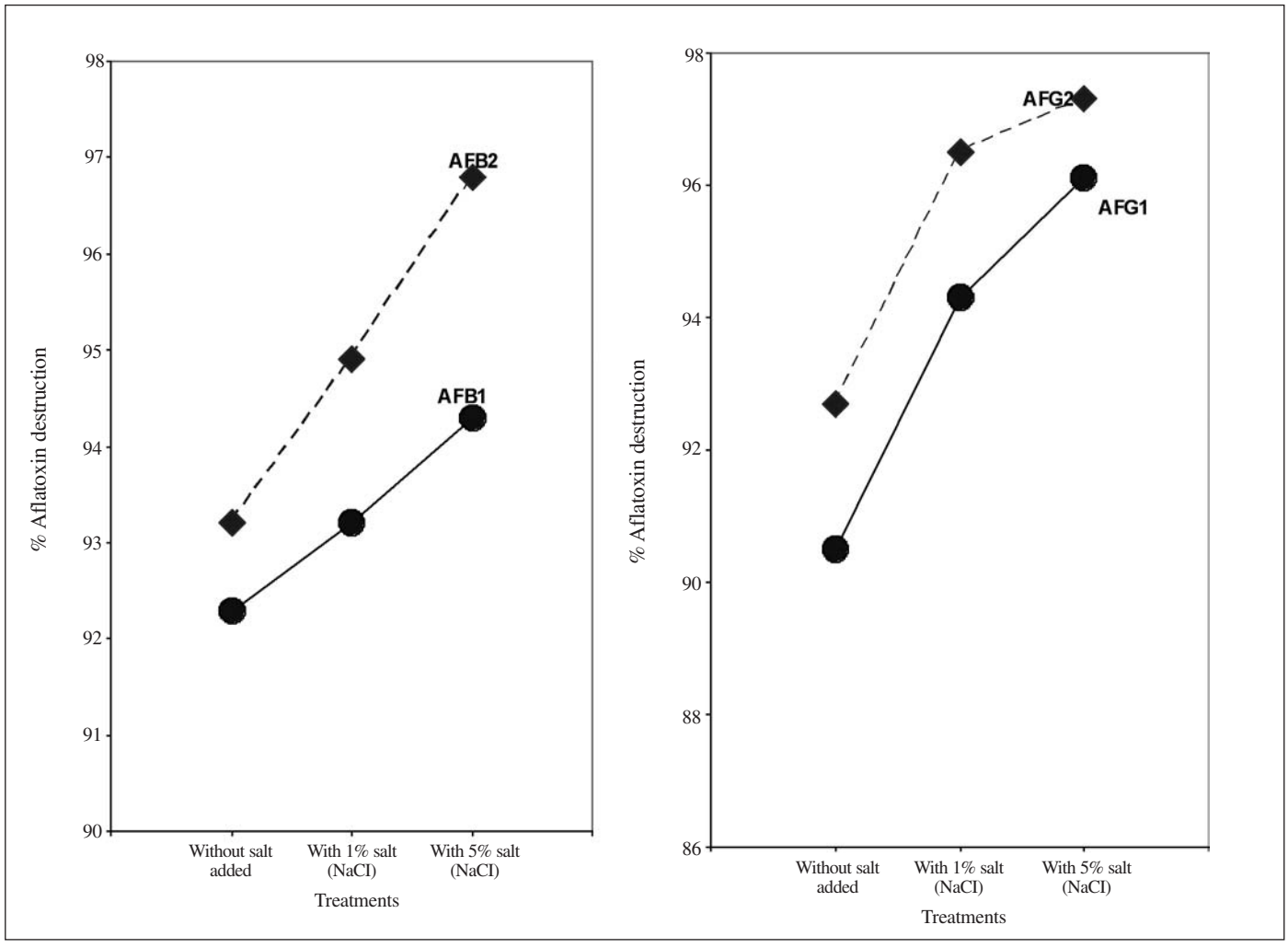

Fig. 2. Effects of popcorn preparation on AFB1, AFB2, AFG1 and AFG2 destruction
From Amra *et al*[19]

Popcorn was prepared by roasting 250 g of each raw corn sample in a stockpot. The first step was made by adding 1 to 5% salt (sodium chloride) with 25-50 ml of frying oil. The second step was done by heating the stockpot for about 5 min. The roasting temperature reached more than 100°C on a direct flame for 2 to 5 min.

Aflatoxins were determined in corn grains samples according to the method described by Gallagher and Latch[33]

and/or vitamin E. After irradiation a highly significant increase in sperm head abnormalities had been found. Protection with vitamin C and/or vitamin E decreased this effect of irradiation. The protective effect of combination of both vitamins was more than that provided by each vitamin alone.

In the same study, both vitamins significantly reduced the frequencies of chromosomal aberration in spermatic germ cells. The protective effect of vitamin E was greater than that afforded by vitamin C. A combined treatment of both vitamins resulted in additional protection over that offered by each vitamin alone. In all animal groups the most frequent aberration found was found in form of translocation from either ring 4 (R IV) or chain 4 (C IV). The percentage of each of these translocations was significantly increased in male mice sacrificed after 15 days post-irradiation.

Conclusions

The overall rationale from reviewing these studies was to clarify that although humans are exposed to numerous environmental agents that can be mutagenic or carcinogentic, there are several hopes for preventing these hazards. Most environmental exposures involve concurrent or sequential exposure to

several agents in air, water, and food. Several studies demonstrated the genotoxic effects of these environmental pollutants and investigated possible protective effects. Potential methods to prevent cellular injury may be those of augmenting endogenous oxidative defenses through the dietary intake of antioxidants such as vitamin C or vitamin E. Non-vitamin antioxidants such as phenolic compounds might also contribute to cellular defence mechanisms. These phenolic compounds are found in many plant species including olive oil, fruits and vegetables. They play a major rôle in controlling oxidative reactions, thus exhibiting anticarcinogenic activities. Prevention of exposure to aflatoxins can be achieved either at the community level (via good agriculture practices) or at the individual level. Probiotic supplement reduces the biologically effective dose of aflatoxin exposure, and may thereby offer an effective dietary approach to prevent the development of liver cancer. It is of utmost importance to recommend reduction in the national exposure standards of exposure to these agents in food. Avoiding exposure to pesticides, reducing aflatoxin levels in food and introducing vaccination programmes for hepatitis viruses would have a greater impact on public health.

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