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Lime (Citrus aurantifolia L.) Juice a potent treatment for the virulent hepatocarcinogen aflatoxin B1 in peanut paste

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ABSTRACT

Objective: This study aimed at providing a method for elimination of aflatoxin B_1 in peanut paste using a natural product. This is of importance given that the occurrence of aflatoxins is generally unavoidable in spite of the use of protective methods, including use of natural products, during storage. **Background:** Aflatoxins are fungal toxins and products of *Aspergillus flavus* and *A. parasiticus* and other less important aspergilli. They include B aflatoxins (B_1, being the most potent hepatocarcinogen known, and B_2) which are produced by both species and aflatoxins G (G_1 and G_2) that are produced by *A. parasiticus* and aflatoxin M_1 and Q_1 metabolites of B_1 and aflatoxin M_2 metabolite of Aflatoxin B_2. Since the discovery of aflatoxins in 1960, after an outbreak of a disease of unknown etiology of turkey in England, the scientific approach towards these toxins concentrated on the protective measures and control of the incidence of these carcinogens. **Methods:** Since the incidence of aflatoxins is generally unavoidable countable attempts were done on the treatment of these toxins in food and feed products. Accordingly, this persuaded testing some easy and familiar culinary approaches to mitigate and/ or perhaps diminish the presence of aflatoxin B_1 ($AFLB_1$) in the commonly consumed peanut (*Arachis hypogaea* L.) paste. Lime juice, in two doses (12.5 and 25 ml), was applied to 25 g peanut paste contained $AFLB_1$ (7.53 ppb) and stored for 1, 3 and 7 days. The test material was then analyzed for $AFLB_1$ using Aflatest® HPLC column of Vicam® of Waters corporation and HPLC Shimatzu® brand. **Results:** The collected data of the test samples reflected a reduction in $AFLB_1$. That is, the addition of 12.5 and 25 ml lime juice, effected 11% and 31% reduction in $AFLB_1$, respectively (for one day storage), 54% and 66%, respectively (for 3 days storage), and 74% and 92%, respectively (for 7 days storage). Sensory evaluation of the two test mixtures of peanut paste and lime juice and the control reflected an acceptability of 83 ± 2; 61 ± 2 and 40 ± 2% for 1: 2, control (0: 1) and 1: 1 mixtures (volume/ weight of the paste and the juice), respectively. **Conclusion:** These results are encouraging for use of lime juice as a treatment for aflatoxin B_1 in peanut paste (preferably 1: 2 mixture) wherever applicable.

KEY WORDS: Aflatoxin B1; Lime Juice; Peanut paste; Treatment

INTRODUCTION

The peanut or groundnut (*Arachis hypogaea* L.) is a leguminous crop of fabaceae (leguminaceae) family [1]. Of 19 million tons of peanut produced annually worldwide 3.5 million tons are counted within the total world protein consumed by man and his animals. Peanuts are consumed as kernels (raw or processed), peanut butter, confectionaries and as a source of edible oil, being known as the fourth of the leading oil crops internationally. However, aflatoxin contamination and elimination in peanuts represents a challenging research issue and global concern [2]. This stands for the importance of the safe use of this demanded food commodity despite the problem of aflatoxins which is associated with unsatisfactory research. These toxins are of fungal origin, produced by *Aspergillus flavus* (B aflatoxins, 1 and 2) and *Aspergillus parasiticus* (B and G aflatoxins, 1 and 2) and their products aflatoxins M (M_1 and M_2) and aflatoxins Q (Q_1 & Q_2) besides other less important aspergilli. Moreover, they are the cause of loss of quarter the total world production of food crops [3]. The natural occurrence of aflatoxins in food crops endangers the health of human and his animals and results in a sizeable cut in returns. They are considered generally unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. Accordingly, the United States Food and Drug Administration (USFDA) has set specifications for acceptable levels of aflatoxins in human food and animal feed which often yield prohibition of commercial lots from the market. That is, the maximum acceptable amount of total aflatoxins in food and feed is 20 ppb (20 μg/ kg) and only 0.5 ppb (0.5 μg/ kg) for aflatoxin M_1 in milk [4]. However, physical methods of aflatoxin detoxification are practiced using irradiation, food fermentation, extracting the solvent, inactivation by a thermal source or microbes, and adsorption. Detoxification by chemical methods is more effective and is achievable by using ammonia and sodium bisulfite. These enable producers and processors together to mitigate the health and economic losses in the industry of peanut [5].

BDA (Butene dialdehyde = *cis* – 2 – butene1, 4 – dial) a product of *Aspergillus niger* F_{25} represents a modern biological method for the detoxification of aflatoxin B_1 in peanut oil *i.e.* it reduced $AFLB_1$ by 78 – 91% [6].

This research focused on the effect of lime (*C. aurantifolia*) juice as a potent remedy for aflatoxin B_1, the most virulent hepatocarcingen ever known. The selection of this test method and process is due to its simplicity and applicability as a daily familiar recipe in the Sudanese cuisine and as an international culinary art and practice. That is, it is therefore consistent with the theme that the occurrence of aflatoxins is generally unavoidable regarding all pre–harvest and post–harvest protective measures. However, a number of eminent international scientists think that the research in aflatoxins is exhausted and that the application of the findings is the answer. Contrary to their belief, is this research and hopefully other future research to come. That is, the present research can be seen as a counterpoint to this line of argument.

MATERIALS AND METHODS

Materials

Peanut paste from some retailers in Khartoum, aflatoxin free; $AFLB_1$ standard inoculum; metallic jug blender; Aflatest® HPLC column of Vicam® of Waters corporation, the science of what's possible ™ .; filter papers; glassware for extraction and cleanup of the test sample, HPLC Shimatzu® brand of the toxicology section, National Chemical Laboratories, Federal Ministry of Health, Khartoum, and methanol HPLC and other miscellaneous glassware and laboratory materials were all exploited in this study. The lime fruits were obtained from a tree in an orchard located in Shambat County in Khartoum North.

Methods

Lime juice (12.5 and 25 ml) was applied to the peanut paste (25 g), found free of aflatoxins, and thoroughly mixed with it and left to stand in a refrigerator in the toxicology lab of the National Chemical Laboratories, Khartoum, for an assigned period of storage 1, 3 and 7 days. However, the aflatoxin quantification procedure was done according to the Vicam® standard method [7] using HPLC Shimatzu® and Aflatest® HPLC column of Vicam® of Waters Corporation.

The analysis of the lime juice, included, total soluble solids, citric acid and ascorbic acid which were determined according to the methods described by the Association of Official American Chemists (AOAC) [8]. Folic acid was determined by microbial assay [9]. While, B vitamins [pantothenic acid (B_5) and folic acid ($B_{9)}$] were evaluated by HPLC [10] with procedures described by AOAC [11].

The sensory evaluation of the test mixtures (1:1, 1:2 and 0:1of lime juice to peanut paste) included twenty semi trained panelists from the staff of the National Food Research Center (NFRC), Khartoum North, Sudan. They were getting coded each test mixture in glass dishes and asked to evaluate odor, taste, after taste and accessibility attributes according to Ihekoronye and Ngoddy [12]. The experiment was carried out in the period (11:00 am to 12:30 pm). The panelists were asked to give the scores from 5 to 1. That is, 5 is the best score and one is the least. The analysis of the data was done by SPSS at 5 % probability level.

RESULTS

Table 1 displays the results of the effect of treatments with two different doses (12.5 and 25 ml) of lime juice during three different storage periods (1, 3 and 7 days) on aflatoxin B_1 in peanut paste. The aflatoxin test records (Table 1) were 6.72 and 5.23 (μg/ kg = ppb) after one day storage and addition of a lime juice dose of 12.5 and 25 ml, respectively. The corresponding records after 3 days storage were 3.49 and 2.54 ppb and that after 7 days were 1.93 and 0.58 ppb, respectively. However, the two test doses of the lime juice (12.5 and 25 ml) effect an aflatoxin B_1 reduction of 0.81and 2.3 ppb for one day storage, 4.04 and 4.99 ppb for 3 days storage and 5.6 and 6.95 ppb for 7 days storage, respectively (Table 2) as compared to the control (7.53 ppb). Nevertheless, the reduction percentage values for 12.5 ml and 25 ml and 1, 3, and 7 days storage, respectively were 11% and 31%; 54% and 66%; and 74% and 92% (Table 1). Moreover, the difference in aflatoxin reduction between the two test doses (12.5 and 25 ml) was 1.49, 0.95 and 1.35 ppb. That is, the % reduction is 184 (1.49 X 100/ 0.81), 24 (0.95 X 100/ 4.04) and 24 % (1.35 X 100/ 5.6) for the difference in the aflatoxin due to the two used doses for a storage period of 1, 3 and 7 days, respectively (Table, 2). The results of the analysis of the lime juice (Table 3) revealed 3.12% total soluble solids (TSS), 8.62% citric acid, 41.73 mg/ 100g ascorbic acid (vitamin C), 4.56% pantothenic acid (vitamin B_5) and 1.75% was reported for folic acid (vitamin B_9). Moreover, the pH (the negative of the logarithm to base 10 of the activity of the hydrogen ion) value was found to be 2.34 (Table 3).

Table 1. Effect of lime juice on aflatoxin B_1 in groundnut paste

Sample	Storage Duration (day)	Aflatoxin (µg/ kg)	Juice (ml)	Reduction (%)	Dose
Control		7.53			
1.1.	1	6.72	12.5	11	0.5 ml/ 1 g
1.2.	1	5.23	25	31	1 ml/ 1 g
2.1.	3	3.49	12.5	54	0.5 ml/ 1 g
2.2.	3	2.54	25	66	1 ml/ 1 g
3.1	7	1.93	12.5	74	0.5 ml/ 1 g
3.2.	7	0.58	25	92	1 ml/ 1 g

Table 2. Aflatoxin reduction of the test dose and storage as compared to control

1 day (ppb)		3 days (ppb)		7 days (ppb)	
12.5	25	12.5	25	12.5	25
0.81	2.3	4.04	4.99	5.6	6.95
Difference	1.49		0.95		1.35
% Difference	184		24		24

Table 3. Results of the analysis of the lime juice from test fruits

Parameter	Amount
Total Soluble Solids (%)	3.12
pH value	2.34
Citric acid (%)	8.62
Ascorbic acid (mg/ 100g)	41.73
Pantothenic acid – B_5 (%)	4.56
Folic acid – B_9 (%)	1.75

Table 4. Sensory evaluation results of the three test mixtures

Quality attribute	Groundnuts sample			$LSD_{0.05}$	SE
	1:1	1: 2	Control		
Odor	42.33^{c} ±2.08	85.00^{a} ±1.00	65.67^{b} ±2.08	3.585	1.036
Taste	42.33^{c} ±0.58	85.33^{a} ±2.08	63.33^{b} ±2.08	3.46	1.00
After taste	42.33^{c} ±0.58	64.33^{a} ±1.53	60.00^{b} ±2.65	3.585	1.036
Acceptability	39.67^{c} ±1.53	82.67^{a} ±2.08	61.00^{b} ±1.73	3.585	1.036

* Means ± SD having different superscript letters in columns and rows are significantly different (P≤0.05).

The results of the sensory test of the two test mixtures and the control are displayed in table 4. The odor test showed 85, 66 and 42% for the 1: 2, control (0: 1) and 1: 1 ratios, respectively. The corresponding readings for taste were 85, 63 and 42%, respectively. The after taste results were 64, 60 and 42% for the mentioned mixtures and the control, respectively. However, the acceptability results were 83, 61 and 40% for 1: 2, control (0: 1) and 1: 1 mixtures, respectively (Table, 4).

DISCUSSION

The two test doses of the lime juice (12.5 and 25 ml) resulted in an aflatoxin B_1 reduction of 0.81and 2.3 ppb for one day storage, 4.04 and 4.99 ppb for 3 days storage and 5.6 and 6.95 ppb for 7 days storage, respectively as compared to the control (Table 1). Moreover the difference in this B_1 reduction between the two used doses is 1.49, 0.95 and 1.35 ppb (μg/ kg), for one, three and seven days storage, respectively. That is, the average difference of the aflatoxin B_1 reduction between the two used lime juice doses is 1.26 ppb which accounts for the effectiveness of an additional fold of 12.5 ml dose used. That is, the 25 ml effected an additional reduction of 184, 24 and 24 % for 1, 3 and 7 day storage, respectively as compared to the other used dose (12.5 ml). This clearly reflects the sound effect of the test dose and the storage period. However, the ratio of the B_1 reduction of the two doses used (12.5 and 25 ml lime juice) are 2.84, 1.24 and 1.24 for one, 3 and 7 days storage, respectively. That is, the effect of the increase in dose diminishes with more storage time. The additional reduction achieved by 25 ml compared to 12.5 ml was 184%, 24 and 24% for 1, 3, and 7 days, respectively. These results collectively infer a strong potency of the lime juice in eliminating the strongest hepatocarcinogen ever known, aflatoxin B_1, in peanut paste. In addition, lime contains a voluminous amount of citric acid (which are responsible for the sour taste), a certain amount of vitamin C and an adequate amount of folic acid (B_9) and pantothenic acid (B_5). Nevertheless, it was reported that samples received 0.5 g/ ml thiamine (Vitamin B_1) reflected a reduced aflatoxin production [14].

It is worth mentioning that voluminous work was done in the detoxification of aflatoxins in food. That is, the effectiveness of ozonation and mild heat against aflatoxins in peanut kernels and flour was studied. Peanut samples were subjected to various ozonation and temperature regimes (25°, 50°& 75°/ 5, 10 & 15 min). Heedless of treatment regimes, aflatoxins B_1 and G_1 exhibited the highest degradation levels and in peanuts as well. The temperature effect was inversely proportional to the treatment time. These results are encouraging in using ozonation at room temperature economically [5].

A diverse group of chemicals has been tested for the ability to degrade and inactivate aflatoxins. A number of these chemicals can react to destroy and degrade aflatoxins effectively but most are *impractical* or potentially *unsafe* because of the formation of toxic residues or the trepidation and denaturation of nutrient content and the organoleptic properties of the product. In addition, two chemical approaches to the detoxification of aflatoxins that have received considerable attention are ammoniation and reaction with sodium bisulfite. Many studies provide evidence that chemical treatment via ammoniation may provide an effective method to detoxify aflatoxin – contaminated corn and other commodities. The mechanism for this action appears to involve hydrolysis of the lactone ring and chemical conversion of the parent compound aflatoxin B_1 to numerous products that greatly decreased toxicity. In this study the detoxification of aflatoxin B_1 definitely include a degradation to other simple compounds which represent a good topic for future research. On the other hand, sodium bisulfite has been shown

to react with aflatoxins (B_1, G_1, and M_1) under various conditions of temperature, concentration, and time to form water soluble products [15]. Moreover, the toxicity of mycotoxins may be greatly affected by the diet constituents that change the normal responses of the species systems to these toxins. A variable array of chemical factors, including nutritional components such as protein, fat, vitamins, and minor elements, food and feed additives (antibiotics and preservatives), as well as other chemicals that mitigate the effects of aflatoxins in animals. This is informative of the role of vitamins in aflatoxin detoxification process that also took place in the study.

A new approach to the detoxification of aflatoxins is the addition of inorganic sorbent materials, *chemisorbents*, such as hydrated sodium calcium aluminosilicate (HSCAS) to the diet of animals. HSCAS strongly binds and restrains aflatoxins in the gastrointestinal tract of animals, and accordingly rendering it biologically unavailable [14].

Forced heated air, liquid nitrogen, hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), sodium oleate, and water spray were tested against aflatoxins in Spanish peanut. Results were significantly better for sodium oleate, water spray, liquid nitrogen, and Hydrogen peroxide (H_2O_2). In addition, H_2O_2, water spray and HCl, in order, gave the best results of the treatments [16]. However, *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC – 705, previously shown to effectively bind to aflatoxin B_1, were subjected to various chemical and physical treatments to examine the effects of these treatments on the binding affinity of these strains towards aflatoxin B_1. The biding ability to aflatoxin B_1 of these strains is enhanced by hydrochloric acid ($P < 0.05$). A slightly weaker effect was also reported by autoclaving or boiling at 100° C in water bath. On the other hand, a reduced effect or no effect was observed when adding ethanol, UV radiation, sonication, alkaline, or altering pH [17]. This goes with what was found in this study *i.e.* the acidic nature of the treatment juice, pH 2.34 (Table, 3). However, the seed extract of Ajowan *[Trachyspermum ammi* (L.) Sprague ex Turrill] showed a maximum degradation of AFG_1 up to 65%. The refined *T. ammi* extract had an edge over the crude extract *i.e.* achieved > 90% degradation of the toxin [18]. In addition, < 10% reduction of $AFLB_1$ was reported within two hours in a medium having 1.5% potassium permanganate, 2.5 and 5% Chloramin B (Lachema) or soda. The same result was obtained with 5% and 60°C heated ammonia or 5% sodium hydroxide, potassium hydroxide or calcium hydroxide, or 50% chromosulphuric acid. Acids used alone had the lowest effectiveness [19]. This reflects the low efficacy of some acids in detoxifying aflatoxins that may be attributed to the nature of the acids themselves.

Sorghum flour contaminated with B – aflatoxins (140 ppb) was extrusion – cooked with aqueous lactic or citric acid at six different concentrations. The aflatoxin reduction is sometimes more effective when using aqueous citric acid (up to 92%), than when using aqueous lactic acid (up to 67%) [20]. The percentage of detoxification produced by citric acid (92%) is equal to that recorded after seven days storage and a dose of 25 ml lime juice in this study. This supports the findings of this test.

The food preservatives sorbic acid (SA) and propionic acid (PA) were assessed with regard to their effect on aflatoxin production in synthetic media or wheat seeds (with 20% moisture). Neither PA nor SA were effective against both fungi and their toxin production, while the combination of BHT (butylated hydroxy toluene) and PA or SA was more effective in controlling aflatoxin production [21]. This likely reflects the inability of some acids to detoxify aflatoxins and a potentiation or additive action.

The effect of some coffee vitamins (thiamine, riboflavin, niacin and folic acid) and some carotenoids against mutations was studied. Results reflect an impeding action of 60% in genetic toxicity of $AFLB_1$ [22], [23], [24] & [25]. This may account for the effect of the B vitamins, found in lime juice also, on $AFLB_1$. However, in a study that dealt with the effect of vitamin C on $AFLB_1$ toxicity in guinea pigs it was concluded that 70% of the test animals fed no vitamin C died within three days of feeding $AFLB_1$ and their livers displayed substantial necrosis and disintegration of the multilopes. On the contrary pigs having 25 mg/ day of vitamin C reflected no death but their livers had symptoms similar to the former group fed no vitamin C [26]. The lime juice used in this study showed 0.42 mg/ g ascorbic acid (Table 3) which might contribute to the detoxification of $AFLB_1$ in the test peanut paste.

The components of the lime juice which includes citric acid (effected a reduction of $AFLB_1$ up to 92% in the cited literature); vitamins B_5 and B_9 that related to vitamin B_1 which was reported to decrease aflatoxin and the pH (2.34) also contributed to the reduction of $AFLB_1$ (as reported in some literature cited); ascorbic acid also prevented death in young guinea pigs gavaged $AFLB_1$ [26]. The cited literature and the findings of this study strongly advocate the use of lime juice as a treatment for $AFLB_1$. This, in addition to its simplicity in use and its widespread availability as a natural product, this lime juice promises significant medical benefits as a known potent antioxidant and immunity supporter.

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