REDUCTION OF THE NEGATIVE EFFECT OF OCHRATOXIN A ON THE ORGANS OF RATS FED OCHRATOXIN A-CONTAMINATED CHOCOLATE BY THE SPICE AFRAMOMUM DANIELLI

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Abstract

Purpose: This work was carried out to study the potential of *Aframomum danielli* spice powder in reducing the negative effect of ochratoxin A in rats fed OTA contaminated chocolate.

Design/methodology/approach: The design comprised 4 x 4 factorial experiments using 20 female albino rats fed with ochratoxin A-contaminated chocolate with average body weights of 180g. The experimental diet consisted of ochratoxin A-spiked chocolate with 20µg/kg of OTA with varying levels of *A. danielli* incorporated at concentrations of 1000ppm, 1500ppm, 2000ppm and 2500ppm respectively. Ochratoxin A-contaminated chocolate without *A. danielli* served as the control. The chocolate was melted and ad-

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ministered in amounts of 2.0 mg/kg BW in 2.0ml of solution of 15% v/v ethanol isotonic saline subcutaneously administered at the base of the ear. The animals were kept inside in groups and they were fed with groats mix. Water was available *ad libitum*. After 10, 15, 20 and 30 days, blood was sampled from the vena cava cranial before feeding in the morning. The actual weights were taken at the start of the experiment and the final weights were taken after slaughter. Formation of free radical oxygen in phagocytes was detected via luminometer. At the expiration of the experiment, all animals were sacrificed after administration of overdose of anaesthetics followed by bleeding. Lung changes were examined. The number of eosinophils was determined in bone marrow smears sampled at autopsy. Ochratoxin A levels in the liver, lungs and spleen were also detected.

Findings: The results indicated changes in body mass development with different levels of ochratoxin A administered. The use of *A. danielli* did not support weight gain. Ochratoxin A levels in the liver, spleen, kidney and lungs were below serum levels by a factor of between 5 and 20, which also depended on the ochratoxin A dose and the *A. danielli* treatments. There was a significant reduction in OTA in all organs of the experimental animals with increase in the administration of *A. danielli*. A significantly high OTA concentration, however, occurred in the kidney.

Practical implications: Since OTA toxicity has been associated with enhanced lipid peroxidation, which could lead to cell damage, the use of *A. danielli* has reduced all the cytotoxic effects on rats fed with chocolate treated with *A. danielli* compared with controls.

Originality/value: The use of A. danielli counteracted the negative effects of ochratoxin A as evidenced in this study.

Keywords: Reduction, Negative effects, *A. danielli*, Ochratoxin A, Chocolate

INTRODUCTION

Under the influence of OTA, the formation of reactive oxygen radicals (ROR) is highly promoted. The radical oxygen formation has been reported to be responsible for cell damage, stress development and other cardiovascular diseases and eventually death. Other problems associated with OTA incidence include nephrotoxicity affecting the kidney. The reduction of OTA in the system is imperative in view of the danger posed to human and animal lives. Experimental and epidemiological evidence supports the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases

(Hallwell *et al.*, 1992). Lipid peroxidation is an autocatalytic free radical-mediated destructive process whereby polyunsaturated fatty acids in cell membranes undergo degradation in the form of lipid hydroperoxides (Sovanian, 1985; Slater, 1984).

Schaaf *et al.* (2002) attributed proximal tubular cell damage caused by OTA to the formation of reactive oxygen species (ROS), which in turn induce oxidative damage in lipids, protein and DNA. Several natural components of diets including ∞-tocopherols and various polyphenols have been reported to exhibit potent antioxidant activities and thus might partly counteract OTA toxicity (Renzulli *et al.*, 2000). In a study on the effects of two diterpenes (kafestol and kahwen) in protecting green and roasted coffee beans against the covalent binding of aflatoxin B1 metabolites to the DNA of rats, Carvin *et al.* (1998) identified diterpenes as potentially chemopreventive agents.

Adegoke *et al.* (2000) also carried out a study on inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *Aframomum danielli*. The superiority of *A. danielli*, a natural antioxidant over two synthetic antioxidants, BHA and BHT, has been reported (Adegoke *et al.*, 2000). This study was carried out with the aim of establishing the counteracting effect of the negative impact of OTA on the organs of female rats fed OTA-contaminated chocolate. The addition of *A. danielli* to the chocolate was also examined for the reduction of formation of oxidation products in the rats *in vivo* as well as the haematological parameters.

MATERIALS AND METHODS

This experiment was designed using a 4 x 4 factorial experiment comprising 20 female albino rats fed with contaminated chocolate with average body weight of 180g.

Experimental diets consisted of chocolate spiked with 20µg/kg of ochratoxin A with varying levels of *A. danielli* at concentrations of 1000ppm, 1500ppm, 2000ppm and 2500ppm. Ochratoxin A-contaminated chocolate without *A. danielli* served as the control. The chocolate was melted and administered in amounts of 2.0ml/kg BW in 2.0ml of a solution of 15% v/v ethanol isotonic saline subcutaneously administered at the base of the ear. The animals

were kept inside in groups and they were fed a groats mix. Water was available *ad libitum*. After 10, 15, 20 and 30 days, blood was sampled from the vena cava cranials before feeding in the morning. The initial weights were taken at the start of the experiment and the final weights were taken before slaughter.

The blood serum collected from the experimental animals was stored at -20^{O}C until use. Ochratoxin A in the blood serum was later determined by means of Enzyme Linked Immunosorbent Assay (ELISA) using Veratox kits produced by Neogen US. The manufacturer's guidelines were used for the analysis. Total leucocyte counts and differential blood counts were determined in accordance with Muller *et al*. (1999). Formation of free radicals in phagocytes was detected in whole blood by applying chemiluminescence amplified by luminol after stimulation with zymosan or lipopolysaccharides in a luminometer using the method of Muller *et al*. (1999). At the expiration of the experiment, all the animals were sacrificed after administration of an overdose of anaesthetic followed by bleeding. Lung changes were examined. The number of oesinophils was determined in bone marrow smears sampled at autopsy after administration of Pappen heime stain. Ochratoxin A levels in the liver, lungs, kidney and spleen were also detected in accordance with Muller *et al*. (1999).

REDUCTION OF FREE RADICAL OXYGEN FORMATION USING CHEMILUMINESCENCE ASSAY

The capacity of *A. danielli* to reduce free radical oxygen formation in rats fed ochratoxin A–contaminated chocolate was assayed by chemiluminescence (Whitehead *et al.*, 1992). Enhanced chemiluminescence signal reagent (Sigma Chemical, US) comprising assay buffer tablets A and B containing luminol p-iodophenol enhancer and perborate oxidant was prepared by adding tablets A and B to the buffer solution. Sign reagent (0.4ml) was added to distilled water (1ml) in a glass cuvette containing a magnetic stirrer. The cuvette was placed in a Parun Wallac Victor 2-chemiluminometer (Sigma Chemical Co.) and the reaction commenced by the addition of 25ml of horseradish peroxidase (4ug/ml in H_2O_2). Lyophilized rat blood (0.5mg/ml) dissolved in PBS (pH 7.4) was added to the cuvette and the time for which light output was suppressed was determined. The determinations were

repeated three times for each sample solution. Comparison was made with a standard cuvette containing different concentrations of Trolox in water.

RESULTS AND DISCUSSION

As shown in Table 1, changes in body mass development were associated with different levels of ochratoxin A administered to the female rats. Treatments of ochratoxin A-contaminated chocolate before feeding to the rats created a reducing effect on the induced negative physiological effects. A reduction in rat weight was observed with increase in *A. danielli* administration on the experimental animals, although there was no significant difference in OTA given to the different *A. danielli* treated groups. Muller *et al.* (1999) reported that body mass development was influenced by ochratoxin A administration, which differs from the results of this study. The use of *A. danielli* may not support weight gain or obesity, as in previous reports (Christoph *et al.*, 2005). Ochratoxin A levels in the liver, spleen, kidney and lung were below serum levels by a factor of between 5 and 20, which also depended on OTA dose and the *A. danielli* treatment. There was a significant reduction in OTA in all the organs of the experimental animals with increased administration of *A. danielli*. A significantly high OTA concentration, however, occurred in the kidneys (Table 1). This report is in agreement with other findings where OTA has been linked with nephrotoxicity (Renzulli *et al.*, 2004) with *A. danielli*, indicating that the major target organ of ochratoxin A is the kidney.

The health effects of *A. danielli*-treated chocolate in ochratoxin A-infected rats is in agreement with the work of Christoph *et al.* (2005), which emphasized that a high consumption of food rich in antioxidants can reduce the risks of cardiovascular and kidney diseases. Since OTA toxicity has been associated with enhanced lipid peroxidation (Schaaf *et al.*, 2002), which could also lead to cell damage, the use of *A. danielli* antioxidant has reduced all these cytotoxic effects on rats fed with chocolate treated with *A. danielli* compared with experimental animals without the antioxidants.

Cowan (1977) reported that naturally-occurring antioxidants have been associated with prevention of oxidative damage induced by the free radicals that cause several human diseases. Phenolic substances in *A. danielli* (a trihydroxy group) have also been proposed as important contributors to

Table 1.
Ochratoxin A concentrations in organs of rats fed ochratoxin A-contaminated chocolate

A. danielli treatment	(ppm)	(g)		(g)	OTA in organμ/kg			
	Initial	Initial weight	Final weight	Liver	Lung	Spleen	Kidney
	OTA/BW						
Control	20,110 ±.11a	122±1.00ab	75.22±0.02b	44.50±0.50a	110±1.00a	29.00±1.00a	138±0.0a
1000	20.05±0.01a	120 ±0.00b	74.40±0.00c	36.50 ±0. 50b	83.00±1.00b	28.00±0.00b	101±1.00c
1500	20.13±00.01a	119±1.00c	73.16±0.00d	31.00±1.00c	41.00 ±1.00c	26.00±0.0b	62.00±0.00c
2000	20.60±0.50a	115± 0.00d	71.31±0.01dc	28.00±1.00c	32.00±1.00c	21.00±1.00c	41.00±1.00d
2500	20.73±0.03a	123±0.00a	70.33±0.07a	6.00±1.00d	11.00±1.00d	20.00±0.00c	35.00±1.00e

a. b. c. d. and e. means having the same letters along the same vertical columns is not significantly different ($p<0.05$)

the total antioxidant capacity (TAC) of *A. danielli* (Adegoke *et al.*, 1998). Further attention has recently been paid to the possible health benefits of dietary phenolic phytochemicals that inhibit antioxidative, antifungal and anti-browning properties (Christoph *et al.*, 2005).

The levels of OTA (ng/g) found in the serum of experimental animals (Table 2) increased from day 0 and reached the highest levels on day 30. With the administration of *A. danielli*, a significant decrease in OTA in the serum was observed in the presence of *A. danielli* and the efficiency of the spice increased from 1000ppm to 2500ppm *A. danielli* (4.4.7). The use of *A. danielli* to suppress (ROR) confirms *in vitro* studies on the use of crude extracts of *A. danielli* as antioxidants in oils (Adegoke *et al.*, 1998, 2000; Fasoyiro *et al.*, 2000). The reduction in free radical oxygen formation and reduction of OTA by antioxidant *A. danielli* has been reported (Adegoke *et al.*, 2000) Reddy *et al.* (1984) reported that antioxidants can have significant effects on reduction of free radical formations which can induce breast and colon cancers. Antioxidant mechanisms have been hypothesized as being responsible for the inhibition of mammary carcinogenesis by dietary kelp in the presence of enhanced antioxidant activity, and reducing lipid peroxide in the liver of treated rats (Maruyama *et al.*, 1991).

In the serum from day 0–30th day, a significant reduction was observed in all *A. danielli* treatments only after the first day. On the 10th day, there was no significant difference in serum OTA when 1500ppm and 2000ppm *A. danielli* powder were used. On day 15, a highly significant reduction in OTA (p<0.05) occurred in all treatments. Ochratoxin A reduction was highest in animals fed OTA-contaminated chocolate and treated

	Aframomum danielli treatment				
Experimental days	Control	1000	1500	2000	2500
8	0.35a0±.21	0.45±0.01a	0.400 ±0.01a	0.38±0.01a	0.33±0.00a
10	305.±.7.07a	245± 0.41b	202±1.41c	200±0.01c	11±1.41d
15	421±0.71a	200±0.00b	162±2.12c	149±1.41d	121±0.71e
20	604 ±1. 41a	595±7.07a	501±1.41b	417±2.12c	204±2.12d
30	1106 ±8.49	851±1.41b	701±1.41c	311±1.41d	111±.1.41e

a. b. c. along the same horizontal line with the same subscripts are not significantly different at p <0.05
± standard deviation

Table 2. Levels of ochratoxin A (µg/kg) in the sera of experimental animals fed with Aframomum danielli treated chocolate

with 2500ppm of *A. danielli*. Some trends were observed after 20 and 30 days respectively. In Table 2, serum OTA of the controls corresponded to levels measured before the administration of the toxin. The level of OTA found in the serum depended on *A. danielli* administered.

The OTA levels in the serum resulting from this study were in agreement with past reports from feeding experiments. Examination of the blood cells (Table 3) showed changes in the haematogen parameters from day 5–20 of the experiment. Compared with animals fed with *A. danielli*-treated chocolate, the control animals exhibited lower total leukocytes, higher lymphocytes and lower neutrophil counts. This might be due to the negative effects of ochratoxin A on haematological parameters (Christoph *et al.*, 2005). The arithmetic mean of the eosinophils counts in the experimental rats was higher, with significant differences becoming apparent with *A. danielli* administration from 1000ppm to 2500ppm (Table 3). Some changes in haematological parameters were observed as a result of ochratoxin A contamination, which indicated immunomodulatory effects, and these changes may depend on the dose and the toxin concentrations (Muller *et al.*, 1999). Administration of *A. danielli* at varying concentrations significantly affected the haematological parameters as a dose of 20µg/kg of OTA resulted in a decrease in oesinophilic cells and an increase in lymphocytic cells together with a reduction in total leucocytes. These findings are in agreement with the results obtained by Muller *et al.* (1999) in their experiment with mice and swine.

Ochratoxin A did not have much effect on the haematological parameters of animals given *A. danielli*-treated chocolate rations only. In an OTA feeding experiment involving doses of 0.3–12.0mg/kg feed, Olojede *et al.* (1993) also found oesinophils in association with human Balkhan endemic nephropathy, which is thought to be linked to OTA exposure. Increased oesinophils in the experimental animals used in this work might be due to an influence of OTA triggered at immunological levels (Li *et al.*, 1998; Lin *et al.*, 1998). As shown in Table 4, there was a strong correlation between OTA administered and the levels found in the liver, lungs, spleen and kidney, and in all these organs the correlation was highly significant with OTA ($p<0.01$). Correlation was significant ($p<0.05$) for the effects of OTA on the initial and final weights of rats fed OTA-contaminated chocolate, and at this probability level, there was also a strong relationship between the liver, the spleen and the kidney (r<

Table 3.
Ochratoxin A concentrations in organs of rats fed OTA-contaminated chocolate

Aframomum		Leucocytes (x 10^3 μl^{-1})				Lymphocytes (10^3 ul^{-1})				Neutrophils (10^3 ul^{-1})				Eosinophils (10^3 ul^{-1})			
Treatments ppm		days				days				days				days			
	OTA	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
Cont.	20	11.6c	10.2de	9.8bc	8.6e	53a	61a	63a	65a	42c	40bc	38d	38d	0.8a	0.9a	1.1a	1.3b
1000	20	12.0b	11.1d	10.7bc	9.2d	49b	53b	54b	57b	44d	41c	39d	38d	0.7b	0.8b	1.0b	1.1c
1500	20	12.3b	12.0c	11.9b	10.4c	49b	52b	50c	52b	46c	49b	40c	44c	0.7b	0.8b	1.0b	1.1c
2000	20	14.8a	13.2b	12.5ab	11.6b	48c	50c	48d	50c	48b	50ab	44b	45b	0.6c	0.7c	9.0c	1.0d
2500	20	14.8a	14.0a	13.00a	12.3a	42d	48d	46d	48bc	56a	52a	50ab	46a	0.5d	0.7c	9.0c	9.3a

a. b. c. d. and e. means having the same letters along the same vertical columns is not significantly different ($p<0.05$); Cont-control

0.0001; 0.0007; 0.0013). This correlation coefficient indicated that the target organs of OTA are the lungs, the spleen, the liver and the kidneys. These findings were in agreement with those of Muller *et al*. (1999) and Varga *et al*. (2000).

EFFECTS OF OCHRATOXIN A ON FREE RADICAL OXYGEN FORMATION IN RATS FED A. DANIELLI-TREATED CHOCOLATE AND UNTREATED OCHRATOXIN A-CONTAMINATED CHOCOLATE

In rats, a suppression of radical oxygen formation as compared with the controls was observed with different levels of *A. danielli* treatments (Table 5). Using luminal-dependent chemiluminescence-expressing early oxidative processes (MDP-H_2O_2 halogenide system), oxidative stress increased from day 1 to day 30, the suppression of radical oxygen formation in rats fed *A. danielli*-treated chocolate. Thompson and Yoon (1984) suggested that polyphenols bind proteins, decrease the activity of digestive enzymes and finally reduce the digestibility and/or adsorption of glucose. Reduction of

	OTA	IW	FW	LIVER	LUNG	SPLEEN	KIDNEY
OTA		0708	0.9885	0.0343*	0.0483*	0.0148*	0.052*
IW			<.0001**	0.607	0.5252	0.3545	0.3299
FW				0.4431	0.6660	0.511	0.4240
LIVER					0.0007**	0.0013**	0.0026
LUNG						>0.0001**	<0.0001**
SPLEEN							<0.0001*

IW – Initial weight; FW –final weight

** Correlation is significant at p<0.05, * correlation is significant at p<0.01

Table 4. Correlation of OTA to the organs of experimental rats

Treatment:					
A. danielli	**0**	**10**	**15**	**20**	**30**
1000ppm	1.20a	19.550±.21b	16.500±.42b	2.600±0.14b	27.80±0.28b
1500ppm	0.40d	15.50±.14c	13.450±.21c	8.85±0.07c	21.44±0.00c
2000ppm	0.10e	11.50±28d	10.50±0.14d	7.30±0.14d	20.1.±0.14d
2500ppm	1.0b	3.950. ±07c	3.10±0.14e	4.15±+0.07e	8.150±0.07e
Control	0.56c	21.350±.07a	23.70±0.14a	30.300±0.28a	33.60±.0.14a

a. b. c. and d. means along the same vertical column with the same alphabets are not significantly different at *p*>0.05

Table 5. Effect of Aframomum danielli on radical oxygen formation species induced by ochratoxin A in animals fed contaminated chocolate

OTA activity by *A. danielli* as shown in this study is not unusual, as several natural components of the diets including ∞-tocopherols and various polyphenols have been reported to exhibit potent antioxidant activities and thus might partly counteract OTA toxicity (Renzulli *et al.*, 2000). Carvin *et al.* (1998), in a study on the effects of two diterpenes (kafestol and kahwen) in protecting green and roasted coffee beans against the covalent binding of aflatoxin B1 metabolites to the DNA of rats, identified diterpenes as potentially chemopreventive agents. Adegoke *et al.* (2000) also carried out a study on the inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *A. danielli*. The superiority of *A. danielli*, a natural antioxidant, over the synthetic antioxidants BHA and BHT has been reported (Adegoke *et al.*, 2000).

CONCLUSION

This study established that the target organs of OTA were the kidney, the spleen and the lungs and OTA negative effects such as radical oxygen formation, which can cause cell damage, were reduced by the chemopreventive activity of *A. danielli*. The use of *A. danielli* as evidenced in this study was not found to support body mass development and thereby did not support obesity. The use of *A. danielli* in the reduction of OTA in any contaminated chocolate is desirable in view of campaigns for the reduction of chemicals in food preservation.

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