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: This experiment was designed in a 4 x 4 factorial experiment using 20 female albino rats fed with contaminated chocolate with average body weight of 180g. Experimental diets consisted of chocolate spiked with 20ug/kg of ochratoxin A with varying levels of A. danielli at concentration of 1000ppm, 1500ppm, 2000ppm and 2500ppm. Ochratoxin A contaminated chocolate without A danielli served as control. The chocolate was melted and administered in amounts of 2.0muKg 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 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 actual weights were taken at the start of experiment and the final weight were taken after slaughter.

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Formation of free radical oxygen in phagocytes was detected using luminometer. At the expiration of the experiment, all the animals were sacrificed after administration of overdose of anaesthetic followed by bleeding. Lungs changes were examined. The number of oesinophils was determined in bone marrow smears sampled at autopsy. Ochratoxin A levels in the liver, lungs, kidney, and the spleen were also detected. 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 n all organs of the experimental animals with increase in the administration of A.danielli. A significantly high OTA concentration, however, occurred in the kidney. Since OTA toxicity has been associated with enhanced lipid peroxidation which could lead to cell damage, the use of A.danielli has been able to reduce all the cytotoxic effects on rats fed with chocolate treated with A.danielli than the other experimental animals without the anti-oxidant.

Keywords: *Reduction, Ochratoxin A, Rats, Aframomum Danielli, Adlibitum*

INTRODUCTION

Under the influence of OTA, the formation of reactive oxygen radicals (ROR) was highly promoted. The radical oxygen formation has been reported to be responsible for cell damages, stress development and other cardiovascular diseases and eventually death. Other problems associated with OTA incidence is Nephrotoxicity affecting the kidney. The reduction of OTA in the system is imperative in view of the danger it poses on human and animal lives. Experimental and epidemiological evidence supports the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases (Halliwell *et al,* 1992). Lipid peroxidation is an autocatalytic free-radical mediated destructive process whereby polyunsaturated fatty acids in cell membranes undergo degradation in form of lipid hydroperoxides (Slater ,1984). Schaal *et al,* 2002 attributed proximal tubular cell damage caused by OTA to the formation of reactive oxygen species (ROS) which in turns induce oxidative damage towards lipids, protein and DNA.. 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,* 2004. .Carvin *et al*, 1998, in a study on the effects of two diterpenes (kafestol and kahwen) prevent green and roasted coffee beans against the covalent binding of aflatoxin B1 metabolites to DNA of rats, 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 *Aframomum danielli*, a natural antioxidant over two synthetic antioxidants, BHA and BHT has been reported (Adegoke *et al,* 2000) This study is 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 on the reduction of formation of oxidation products in the rats *in vivo* as well as the haematological parameters of the rats

MATERIALS AND METHODS

This experiment was designed in a 4 x 4 factorial experiment using 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 concentration of 1000ppm, 1500ppm, 2000ppm and 2500ppm. Ochratoxin A contaminated chocolate without *A danielli* served as control. The chocolate was melted and administered in amounts of 2.0ml/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 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 experiment and the final weight were taken before slaughter

The blood serum collected from the experimental animals were 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 United States of America. The manufacturers' guidelines were used

for the analysis. Total leucocytes counts and differential blood counts were determined according to Muller *et al*, 1999. formation of free radical in phagocytes was detected in whole blood by applying chemiluminscence 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 overdose of anaesthetic followed by bleeding. Lungs 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 the spleen were also detected in n accordance with Muller *et al* 1999

REDUCTION OF FREE RADICAL OXYGEN FORMATION USING CHEMILUMINISCENCE ASSAY

The capacity of *Aframomum danielli* to reduce free radical oxygen formation in rat fed ochratoxin A –contaminated chocolate was assayed by chemiluminiscence (Whitehead *et al*, 1992). Enhanced chemiluminiscence signal reagent (sigma chemical,USA) 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) were added to the cuvette and the time for which light output was suppressed was determined. The determinations were repeated three times fot each sample solution. Comparison was made with a standard cuvette containing different concentrations of Trolox in water

RESULTS AND DISCUSSION

In table1, changes in body mass development was associated with different levels of ochratoxin A administered on the female rats/ Treatments of ochratoxin A contaminated chocolate before feeding it to the rats created a reducing effects on the induced negative physiological effects on the rats. A reduction in weights of the rats was observed with increase

in *Aframomum danielli* administration on the experimental animals although thee 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 is different from what was discovered in this study. The use of *A. danielli* may not support weight gain or obesity as 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 increase in the administration of *A. danielli*. A significantly high OTA concentration however, occurred in the kidneys (tale, 1). This report is in agreement with other findings where OTA has been linked with Nephrotoxicity (Renzulli *et al*, 2004) *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 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 antioxidant can reduce the risks of cardiovascular and kidney diseases. Since OTA toxicity has been associated with enhanced lipid peroxidation (Schaal *et al*, 2002) which could also lead to cell damage, the use of *A.danielli* antioxidant has been able to reduce all these cytotoxic effects on rats fed with chocolate treated with *Aframomum danielli* than in 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 causes several human diseases . Phenolic substances in *Aframomum danielli* (a trihydroxy group) has also been proposed as an important contributor to the total antioxidant capacity (TAC) of *Aframomum danielli* (Adegoke *et al*, 2000). Other attentions have recently been paid to the possible health benefits of dietary phenolic phyttochemicals that inhibit antioxidative, antifungal and antibrowning 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

Table 1 Ochratoxin A concentrations in organs of rats fed ochratroxin – A contaminated chocolate

				OTA in organμ/kg			
(ppm)		(g)	(g)				
A.danielli treatment	Initial OTA/BW	initial weight	Final weight	Liver	Lung	Spleen	Kidney
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 same vertical columns are not significantly different ($p<0.05$)

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

Experimental days	Control	*Aframomum danielli* treatment 1000	1500	2000	2500
0	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 same horizontal line with same subscripts are not significantly different at $p < 0.05$
± standard deviation.

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

Table 4: : correlation of OTA to the organs of experimental rat-

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

Treatment:	Experimental days				
A. damIelli	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 same vertical column with same alphabets are not significantly different at $p>0.05$.

administration of *A.danielli*, a significant decrease in OTA in the serum was observed. was enhanced 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 has been hypothesized as being responsible for the inhibition of mammary carcinogenesis by dietary kelp in the presence of enhanced antioxidant activity and reducing lipid perooxide in liver of treated rats (Fasoyiro *et al*., 2000)

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. Ochrtaoxin A reduction was highest in animals fed OTA contaminated chocolate and treated 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 the OTA found in the serum depended on *A.danielli* administered. The OTA levels in the serum resulting from this study was 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 neutrophils counts. This might be due to the negative effects of ochratoxin A hematological 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 hematological 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 hematological 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, 1999) in his experiment with mice and swine. Ochratoxin A did not have much effects 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, Tata et al, 1998, 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 the immunological levels (Li et al, 1998, Lin et al, 1998). In table 4, there was a strong correlation between OTA administered and the levels found in the liver, lungs, spleen and the kidney and in all these organs the correlation were highly significant with OTA ($p<0.01$). Correlation was significant ($p<0.05$) for the effects of OTA on initial and final weights of rats fed OTA contaminated chocolate and at this probability levels, there was also a strong relationship between the liver, the spleen and the kidney ($r< 0.0001$; 0.0007, 0.0013). This correlation coefficient indicated that the target organs of OTA are the lungs, the spleen, the liver and the kidneys. This findings was in agreement with (Muller *et al* , 1999; Varga *et al*, 2000).

EFFECTS OF OCHRATOXIN A ON FREE RADICAL OXYGEN FORMATION IN RATS FED A.DANIELLI TRADED 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 cheminluminiscncee 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 *Aframomum danielli* treated chocolate. Reduction of 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 ben reported to exhibit potent antioxidatnt activities and thus might partly counteract OTA toxicity (Renzulli *et al,* 2004).Carvin *et al,* 1998, in a study on the effects tof two diterpenes (kafestol and kahwen) prevents green and roasted coffee beans against the covalent binding of aflatoxin B1metabolites to DNA of rats, 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 *Aframomum danielli*, a natural antioxidant over two synthetic antioxidants, BHA and BHT has been reported (Adegoke *et al,* 2000

CONCLUSION

This study established that the target organ of OTA was the kidney, the spleen and the lungs and OTA negative effects r such as radical oxygen formation which can cause cell damage was reduced by the chemo preventive activity of *Aframomum 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 reduction of OTA in any contaminated chocolate is desirable in view of the campaigns in the reduction of chemicals in food preservations.

ACKNOWLEDGEMENT

Authors wish to acknowledge the permission of Professor G.O.Iremiren for permission to publish this paper

BIOGRAPHY

Dr Aroyeun is a Chief Research Officer in the Crop Processing and Utilization of the Cocoa Research Institute of Nigeria, Ibadan He is an erudite scholar in the area of processing, quality control and Microbiology/safety of foods. He is a renowned scholar in the fields of mycotoxins, especially in reduction of ochratoxin A in cocoa and cocoa products

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