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NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis. I. Study design and clinical outcomes

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(*Received 18 December 2006; revised 16 May 2007; accepted 17 May 2007*)

Abstract
A 3-month double-blind and placebo-controlled, phase IIa clinical trial was conducted in Ghana to investigate the safety, tolerance and aflatoxin-sorption efficacy of dietary NovaSil (NS). Volunteers (507 subjects) were clinically screened to evaluate their general health, pregnancy status and blood AFB_1–albumin adduct levels. Of these subjects, 177 were randomly assigned to three groups: high-dose (HD), low-dose (LD) and placebo-control (PL) groups receiving 3.0, 1.5 and 0 g NS day^{-1} in capsules. Trained study-monitors supervised NS capsule administration to participants and recorded side-effects daily. Physical examinations were performed monthly. Blood and urine samples were collected for laboratory analysis. Approximately 92% of the participants (162 of 177) completed the study and compliance rate was over 97%. Overall, 99.5% of person × time reported no side-effects throughout the study. Mild to moderate health events (~0.5% of person × time) were recorded in some participants. Symptoms included nausea, diarrhea, heartburn and dizziness. These side-effects were statistically similar among all three groups. No significant differences were shown in hematology, liver and kidney function or electrolytes in the three groups. These findings demonstrate that NS clay is apparently safe and practical for the protection of humans against aflatoxins in populations at high risk for aflatoxicosis.

Keywords: *NovaSil clay, aflatoxin, enterosorbent, safety, clinical outcomes*

Introduction

Aflatoxins (AFs) are harmful by-products of mold growth produced primarily by the fungi *Aspergillus flavus* and *A. parasiticus* (Busby and Wogan 1984; Kurtzman et al. 1987). The naturally occurring AFs (e.g. B_1, B_2, G_1, and G_2) have been characterized as hazardous contaminants that occur either separately or concurrently in a variety of foods consumed by humans and animals (CAST 2003). Aflatoxin B_1 (AFB_1) has been characterized as genotoxic, immunotoxic and hepatocarcinogenic (Busby and Wogan 1984; Flaherty and Payne 1997; IARC 1993, 2002; Hinton et al. 2003; Turner et al. 2003; Wang and Groopman 1999; Jiang et al. 2005). Humans and animals with acute aflatoxicosis typically experience symptoms including jaundice, low-grade fever, GI bleeding, edema, depression, anorexia, diarrhea, fatty liver, ascites, abdominal pain and, potentially, liver failure and death based on dose (Ngindu et al. 1982; CAST 2003). Previous reports have revealed a strong dose–response relationship between exposure to AFs and growth impairment, particularly stunting (a reflection of chronic malnutrition) and underweight (an indicator of acute malnutrition), as seen among children in Benin and Togo, West

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ISSN 0265–203X print/ISSN 1464–5122 online © 2008 Taylor & Francis
DOI: 10.1080/02652030701458105

Africa (Gong et al. 2002; 2003; Williams et al. 2004).

One of the most severe outbreaks of acute AF poisoning occurred recently in Kenya (East Africa) and was linked to the consumption of meals prepared from locally grown and poorly stored maize contaminated with AFs at levels as high as 8000 ppb. The outbreak claimed 125 lives, about 39.4% of the 317 cases reported from January to 20 July 2004 (CDC 2004; Lewis et al. 2005; Azziz-Baumgartner et al. 2005). Previous reports have also indicated that AF contamination of foods intended for humans, particularly maize and groundnuts, constitute major food safety problems in Ghana due to poor handling and storage. For instance, Awuah and Kpodo (1996) reported AF levels ranging from 5.7 to 22 168 ppb in market groundnut samples in Ghana. Another study in Ghana indicated that total AF levels in ''kenkey'' (a common maize-based meal) ranged from 6.2 to 196.1 ppb, with a mean value of 50.9 ppb, in 94% of the samples collected (Jesperson et al. 1994).

The most common effect associated with chronic AFB_1 exposure is the increased incidence of hepatocellular carcinoma (HCC) (Roebuck and Maxuitenko 1994; Shupe and Sell 2004). AFB_1 is implicated as a major risk factor in the etiology of HCC, particularly in tropical areas of sub-Saharan regions of Africa, Southeast Asia and South America (Wogan 1992; IARC 1993, 2002; CAST 2003). The carcinogenic potency of AFB_1 in individuals positive for hepatitis B virus (HBV) surface antigen (HBsAg) is about 30-fold higher compared to individuals who are negative for HBsAg (JECFA 1998; Henry et al. 2002). Therefore, it is imperative to develop and implement intervention strategies that are effective against AFs in the diet, particularly for humans at high-risk for aflatoxicosis or AF-synergized risks, such as HCC from HBV. Given the estimate that 80% of HCC cases occur in developing countries (Wild and Hall 2000), preventive strategies should be economically feasible, culturally acceptable and sustainable. A novel approach that meets the above criteria is dietary inclusion of NovaSil clay that can preferentially bind AFs in the gastrointestinal tract and reduce toxin bioavailability to the blood, liver and other organs (Phillips 1999; Phillips et al. 2002).

NovaSil (i.e. NS) is a naturally occurring, processed calcium montmorillonite clay used as an anti-caking agent. Equilibrium adsorption isotherms and molecular modeling studies have shown that NS preferentially binds AFs that contain a planar ketolactone system (Grant and Phillips 1998; Phillips et al. 2002). Previous short-term studies in rodents, chicks, turkey poults, pigs, lambs, dairy goats and cattle, confirmed that dietary inclusion of NS results in significant protection from AFs (Phillips et al. 1988; Mayura et al. 1998; Kubena et al. 1991; Harvey et al. 1991a,b; Lindemann et al. 1993; Smith et al. 1994; Pimpukdee et al. 2004). In all these studies, no observable adverse effects were reported following dietary ingestion of NS clay (Phillips 1999; Phillips et al. 2002). In developmental toxicology studies in Sprague–Dawley (S-D) rats fed dietary NS at concentrations as high as 2% (w/w) throughout pregnancy, no NS-related maternal or fetal toxicity was detected and no significant changes occurred in trace metal bioavailability in a variety of maternal and fetal tissues (Mayura et al. 1998; Wiles et al. 2004). In a recent long-term study (6.5 months) in S-D rats treated with 0.25–2% (w/w) dietary NS clay, there were no dose-dependent or NS-related adverse effects on body weight gains, relative organ weights, gross and histological appearance of major organs, or hematological and serum biochemistry parameters. Additionally, levels of essential nutrients including vitamins A and E, and the micronutrients Fe and Zn were unaffected (Afriyie-Gyawu et al. 2005).

Given the safety and efficacy of NS, as demonstrated in a variety of animal models, it was hypothesized that NS-based intervention would be beneficial for the treatment of humans who are frequently exposed to high levels of aflatoxins and at risk of aflatoxicoses. As a precursor to a human clinical trial with NS in Ghana, a short-term (2 weeks) double-blind phase I study was conducted to: (1) evaluate the safety and tolerance of NS capsules in 50 healthy human volunteers; and (2) establish optimal protocols for human intervention studies. After 14 days of NS ingestion (1.5 and 3.0 g day^{-1}), participants' compliance (99.1%) was excellent; physical examination results, urinalysis, serum biochemical and hematological parameters were unaffected; serum minerals and vitamins A and E levels were not significantly different from baseline values (Wang et al. 2005). Apart from its demonstrated safety, the 3.0-g dose of NS per day used for the clinical intervention trials, was extrapolated from previous in vitro and in vivo studies, including Pimpukdee et al. (2004), showing that 0.25% NS in the diet was the minimal effective dose that protected chicks from AF toxicity. This study provided the basis for the current Phase IIa human intervention study at Ejura-Sekyedumase district (ESD) of the Ashanti region of Ghana, West Africa.

The ESD of the Ashanti region was chosen as the intervention study site based on a report that AFB_1–albumin adducts and aflatoxin M_1 metabolites were detected in 100% of 140 sera samples and 91.2% of 91 urine samples collected from study participants in the area (Jolly et al. 2006). These findings are supported by Wild et al. (1992) in that a majority (75–100%) of people from East and West African

countries test positive for blood AFB_1–albumin adducts. In this report, our main objective was to evaluate the safety of NS when administered to humans for the management of aflatoxicoses by: (1) determining potential adverse effects of NS in human subjects over a 3-month period and (2) establishing a basis and dosimetry for long-term (Phase IIb or Phase III) studies in human subjects.

Materials and methods

Procurement of test article (NovaSil)

NovaSil clay (NS) was obtained from Engelhard Chemical Corporation (Iselin, NJ, USA). Initially, the NS clay used for the study was evaluated for potential environmental contaminants including polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs) and heavy metals. Evaluation of NS for the US Environmental Protection Agency (USEPA) priority dioxins/furans (17) was performed by Columbia Analytical Services (CAS), Inc. (Houston, TX, USA). Standardized procedures of USEPA methods were used for sample preparation, cleanup and analysis with high resolution capillary column gas chromatography/high resolution mass spectrometry (USEPA Method 1613B). Also, the NS clay was analysed for heavy metals (e.g. As, Cd, Hg, and Pb) by CAS, Inc. (Kelso, WA, USA). Metal analysis procedures followed standard USEPA protocols (e.g. Method 6010B and 7471A). NS clay (containing acceptable levels of contaminants) was sent to College Pharmacy (Colorado Springs, CO, USA) for encapsulation under sterile conditions in a setting with good manufacturing practices. Capsules, with the same size, shape and colour, were formulated to contain 500 mg NS, 250 mg NS or placebo, based on previous dosimetry protocols (Wang et al. 2005). The capsules were then sterilized in sealed plastic containers, approximately 180 capsules/container, by electron beam irradiation (National Center for Electron Beam Food Research, Texas A&M University). A target dose range of 8.2–9.4 KGy was applied and followed protocols similar to those used for sterilizing human foods in the USA. All other chemicals, reagents and solvents used were obtained commercially at the highest purity available.

Study site and screening procedures

Ejura-Sekyedumase district (ESD) is one of the 21 districts in the Ashanti Region, Ghana. Its climatic conditions and soil fertility favour cropping and approximately 76% of the populace engages in agriculture. The main crops produced and consumed in this area include maize, groundnuts, yams, cassava, cotton and tobacco (Adu and Mensah-Ansah 1995; District Health Directorate, Ejura/Sekyedumase 2005). The District Health Director and other health personnel coordinated the community entry process and introduced the research team to the leaders and residents of six communities, which constitute over half of the district population. Prior to the study, four of these communities had established AF baseline data and demographic information (Jolly et al. 2006). The other two communities are in the Ejura sub-district, which has the highest population in the entire area.

Leaders of each community organized a meeting for the investigators to define the purpose, duration, time frame, responsibilities of potential participants and monitors and other aspects of the study, and to allow the residents time for questions and answers. After the community entry and sensitization process, a total of 507 residents from the six communities volunteered to participate in the study. Following explanation of the informed consent document, volunteers signed it and completed a survey instrument comprising health history and food frequency questionnaires. Subjects were then given coded identification numbers and were provided sterile covered cups to collect specimens of their first urine in the morning. On the sample collection day, volunteers went through physical examinations performed by on-site physicians. Also, they provided urine specimens for urinalysis and human chorionic gonadotropin (HCG) test to determine pregnancy status, and donated a total of 15 ml blood in two tubes (5 ml in one tube and 10 ml in another) for AF exposure analysis, liver and kidney function, and hematology. The 5-ml aliquots of the blood specimens were sent to the Ejura District Hospital, Ashanti Region, Ghana for hematological analysis (mainly WBC count, hemoglobin and hematocrit). Aliquots of sera collected from the 10-ml blood specimens were used for liver and kidney function tests at the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Accra. The remaining serum samples were stored at $-20\,^{\circ}C$ and later shipped to Texas Tech University (TTU) to determine AFB_1–albumin adduct levels of each volunteer.

Inclusion criteria, participant recruitment and sample size rationale

Individuals (males and females) who qualified as study subjects met the following criteria: healthy status based on physical examination results, age 18–58 years, intake of corn and/or groundnut-based foods at least 4 times a week, blood AFB_1–albumin adduct levels > 0.5 pmol AFB_1 per mg albumin

adducts (LOD = 0.05 pmol AFB_1 per mg albumin), no history of chronic disease(s), no use of prescribed medications for chronic or acute illness, non-pregnant and/or non-breastfeeding females, normal ranges of hematological parameters, liver and renal function indicators (blood and urine parameters), and a signed consent form. Subjects with abnormal liver function values (ALT) were excluded from the study. Therefore, no acute hepatitis patients were included in the study, regardless of their HBV status (HBsAg + or HBsAg–). Of the 507 volunteers, there were 302 subjects who met all of the inclusion criteria other than the AF–Alb adduct levels. A target population of 180 subjects was selected from this group based exclusively on their adduct levels. To determine if NS was effective and further evaluate its safety, a total sample size of 180 subjects (with 60 per treatment group) was chosen based on the standard 100–300 subjects required by the US NIH Guidelines (2006) for Phase II clinical studies. At the beginning of the trial, two females and one male dropped out leaving 177 (101 males, 76 females) who were finally recruited as study participants.

Overall study design and procedures

The overall study design followed the guidelines for a randomized, double-blind, placebo controlled Phase II clinical trial. Study protocol was approved by both the TAMU Institutional Review Board (IRB) and NMIMR-IRB for Ethical Clearance in Ghana. Screening of volunteers was initiated in September 2005. The trial started in December 2005 and was completed in April 2006 (including a one-month post-trial follow-up). The subjects who met the recruitment criteria were randomly divided into three study groups (60/group) based on serum AFB_1–albumin adduct levels. The first three subjects with the highest AF exposure levels were randomly divided into the three groups, followed by participants with the next three highest exposure levels and so on until all the 180 subjects were divided. As a double-blinded study, the participants, on-site doctors, nurses and all other field workers had no knowledge of the contents of the capsules. To ensure maximum compliance to the defined treatment regimens, maintain blinding to weights of NS capsules and participant well-being, trained study monitors delivered the capsules daily, witnessed ingestions and recorded any symptoms that subjects might have experienced. Physical examinations were performed monthly to evaluate the general health status of study subjects. Urine and blood samples from each participant were collected at the beginning (time 0), one, two and three months of NS ingestion. After three months of capsule ingestion, subjects were monitored without NS treatment for another month. At the end of the fourth month, participants underwent final physical examinations and blood and urine specimens were collected. Figure 1 represents a flow chart of the overall study design delineating the main procedures employed for this research. EDTA-blood samples were sent to Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana for hematological analysis utilizing an Auto-Analyzer, Sysmex KX-21 (Sysmex Corporation, Kobe, Japan). Serum biochemical analysis was performed at NMIMR with a chemistry auto-analyzer (Eos Bravo Plus; Hospitex Diagnostics, Italy). An Electrolyte (Na/K) auto-analyzer (Humalyte; Human Diagnostic, Germany) was used for electrolyte analysis at NMIMR. Portions of urine and serum samples were shipped to TAMU and TTU for efficacy evaluations, the results of which will be published separately. Briefly, urine samples were analysed for an acute biomarker of AF exposure, AFM_1, following protocols reported by Groopman et al. (1992), with modifications of Sarr et al. (1995) and Wang et al. (1999). AFB_1–albumin adducts, another biomarker delineating long-term exposure to AFs, was measured in the serum using protocols reported by Wang et al. (1996).

Treatment protocols

Participants were given ID numbers and randomly assigned to one of the following three treatment groups: high-dose (HD), low-dose (LD) or placebo (PL), implying that they would take two capsules containing 500 mg NS, 250 mg NS and 250 mg placebo, respectively, three times day–1 (before meals and with at least 100 ml of water) over a period of three months. In total, the HD and LD groups received 3.0 and 1.5 g NS day^{-1}, respectively. As a safety precaution, 3.0 g NS was selected as the highest dose since it represented the MED (minimal effective dose) of NS for AFs based on previous animal studies. Dose selection for this study was also based on extrapolations from previously published dosimetry data in animal models (Phillips 1999; Phillips et al. 2002) and NS levels used for a short-term human study in the USA (Wang et al. 2005). The HD (3.0 g NS day^{-1}) represents approximately 0.25% NS (w/w) of the estimated amount of food (1200 g) consumed daily by an average Ghanaian. Furthermore, up to 2% NS (w/w) in the diet, which is eight times higher than the HD level in this study, exhibited no significant adverse effects in rodents following 6.5 months of exposure (Afriyie-Gyawu et al. 2005).

Toxicity and adverse effect monitoring

To monitor potential toxicity of NS ingestion, a symptom checklist was developed and included with

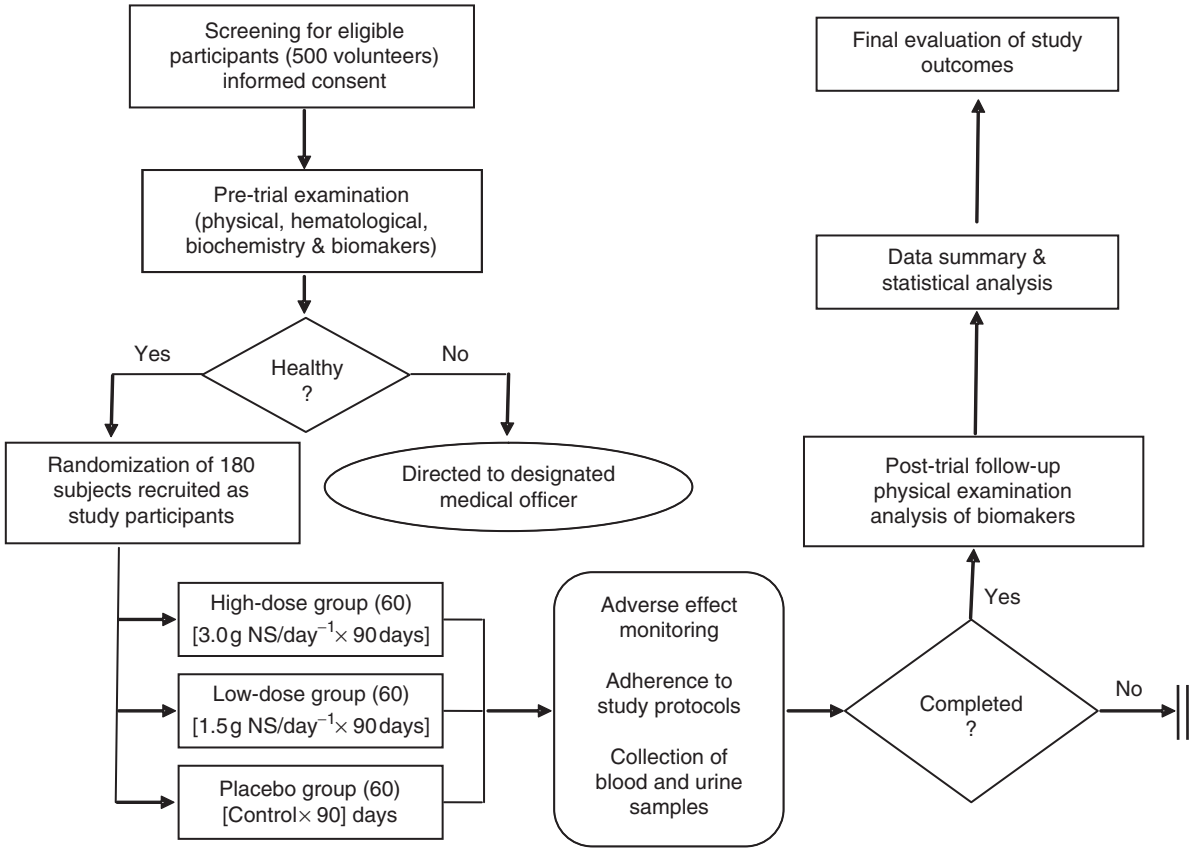

Figure 1. Flow chart delineating the study design and procedures for a 3-month clinical intervention trial with NS in Ghana.

the Daily Diary Worksheet (DDW) as an assessment tool. Study monitors recorded any adverse events and were required to report any health problem to the supervisor and/or on-site physician. Physicians on the investigative team reviewed the DDW every 2 weeks. Symptoms were assessed based on the following criteria:

> Mild (grade 1), slightly bothersome and relieved with symptomatic treatment;
> Moderate (grade 2), bothersome and interfered with activities and only partially relieved with symptomatic treatment;
> Severe (grade 3), prevented regular activities and not relieved with symptomatic treatment.

Whenever symptom(s) were reported, physical examinations and laboratory analysis were performed for verification, if necessary, during the study. Subjects were treated by the on-site physician and allowed to continue the study if symptoms were not perceived to be related to NS ingestion. If symptoms were linked to NS capsules, the participant was treated and asked to discontinue capsule ingestion. The on-site physician had access to an Adverse Event Report document, developed under US NIH Guidelines, for reporting any adverse effects to the investigators and the IRBs of NMIMR and TAMU.

Data management and statistical analysis

All data were entered by the data management team at NMIMR using the coded identification numbers of the subjects. Personnel, other than the investigators, had no access to the names of the participants. All data from the questionnaires, clinical reports, DDW for ingestion and toxicity monitoring and adverse event episodes were entered and managed using Microsoft Excel software. Upon completion of the data entry process, two investigators independently reviewed the recorded data to ensure accuracy.

To show the safety of NS capsule ingestion, the statistical evaluation focused on the comparisons among different treatment levels and different timepoints. Means, standard deviations and medians were calculated for each parameter, and the values of parameters are expressed as mean $\pm$ SD unless otherwise stated. To the parameters that were

normally distributed, two-factorial ANOVA and Bonferroni procedures were used to compare significant differences between means of different treatment arms and times. Chi-square test was used for analysis of adherence and rate of side-effect/toxicity data. To the parameters that were not normally distributed, Kruskal–Wallis test or Wilcoxon rank sum test were used to compare the difference among different treatment groups and different time points. A *p*-value of less than 0.05 (two-tailed) was considered significant. All analyses were done with SAS software version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

Dioxins/furans and metal analyses

Among the 17 USEPA priority PCDDs/PCDFs, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) and octachlorodibenzo-*p*-dioxin (OCDD) were the only two contaminants in NS present above the limits of detection (LODs = 1.11 parts per trillion (ppt) for HpOCDD and 1.91 ppt for OCDD). The mean concentrations of HpCDD and OCDD in NS clay were 4.42 and 23.74 ppt, respectively. Applying the toxic equivalent factors (TEFs) (WHO 1998), the toxic (or TCDD) equivalent (TEQ) values of these dioxins were calculated to be 0.0442 and 0.00237 ppt for HpCDD and OCDD, respectively, with a combined TEQ of 0.0466 ppt in NS. In the HD treatment, the 3.0-g of NS provided a TEQ of 0.1397 pg day^{-1}. According to WHO standards, the tolerable human intake (THI) of TCDD is 2.3 pg kg^{-1} BW day^{-1}, translated to be 161 pg day^{-1} for a 70-kg man and 138 pg day^{-1} for a 60-kg woman (developed by WHO, reported by Van den Berg et al. 1998). Based on these values, the TEQ from 3.0 g NS day^{-1} would be approximately 1000 and 1100 times lower than the daily WHO–THI standards for an average woman and man, respectively. Heavy metals, such as As, Cd and Pb, had levels that ranged from 7- to 80-fold lower (data not shown) in 3 g NS day^{-1} compared to the standard recommended values (JECFA 1998). Hg was found to be below the detection limit (LOD = 0.009 mg kg^{-1} NS) based on the analytical method used.

Screening process and demographic information of study subjects

All 507 volunteers screened were positive for serum AFB_1–albumin adducts (range: 0.1–4.7 pmol AFB_1 mg^{-1} albumin). Table I delineates the demographic characteristics of the subjects enrolled in the study. Initially, we selected a total study population of 180 based on defined inclusion criteria and randomly divided them into three groups (60 per group) – HD, LD and PL – based on participants' serum AFB_1–albumin adduct levels. Three subjects (one from LD and two from PL groups) were

Table I. Demographics and physical parameters.

Demographic characteristics	Treatment group		
	High dose	Low dose	Placebo
Participants	60	59	58
Gender			
Male	34	31	37
Female	26	28	21
Community			
Dromankoma	13	12	20
Ejura Group	10	8	9
Nkwanta	6	8	5
Hiawoanwu	18	15	12
Kasei	3	5	3
Kotokoli Line	10	11	9
Age (years)[a]	38.6 ± 13.0	37.3 ± 11.8	36.5 ± 10.8
Body weight (kg)[a]			
Before trial	60.9 ± 10.2	59.8 ± 11.0	62.6 ± 9.8
After trial	61.4 ± 10.1	62.9 ± 10.3	61.3 ± 10.9
Systolic blood pressure (SBP) (mmHg)[a,b]			
Before trial	126.4 ± 22.6	121.9 ± 23.1	129.9 ± 24.5
After trial	121.0 ± 20.0	112.7 ± 15.0*	117.4 ± 19.8*
Diastolic blood pressure (DBP) (mmHg)[a,b]			
Before trial	79.1 ± 15.8	77.1 ± 15.2	81.5 ± 14.7
After trial	79.0 ± 16.2	75.0 ± 11.3	77.2 ± 13.7

[a]Mean ± SD; [b]normal values are 120/80 for SBP/DBP; $p < 0.05$, $^*p < 0.01$ compared to baseline values.

removed on the day treatment was initiated – two females became pregnant and one male opted out because of a new job. Physical parameters, such as age, body weight and diastolic blood pressure, were not significantly affected after 3 months of NS ingestion. At the end of trial, the mean values of systolic blood pressure (SBP), although no clinical significance, were significantly reduced ($p < 0.01$) for LD (112.7 ± 15.0 mm Hg) and PL (117.4 ± 19.8 mm Hg) compared to baseline values of 121.9 ± 23.1 and 129.9 ± 24.5 mm Hg, respectively. The SBP of the HD group was unchanged.

Completeness of study and compliance with study protocol

The percentages of study participants who completed the entire 3-month trial were 90.0, 89.8 and 94.8% for HD, LD and PL, respectively, and the overall number of subjects who completed the study constituted 91.5%. A total of 15 (six from HD, six from LD and three from PL) of the 177 study subjects did not complete the study. In terms of compliance, 97.4, 96.4 and 98.6% of participants in HD, LD and PL groups, respectively, adhered to the NS-treatment regimen according to the study protocol. The overall adherence (number of times capsules were taken) among the participants, whether or not they completed the study, was over 97%. Data representing participant compliance and study completion are summarized in Table II.

Health incidences and adverse events

Symptoms reported to the study monitors by participants are indicated in Table III. The NS dose levels (1.5 or 3.0 g day^{-1}) were tolerable for the participants in the HD and LD groups. Symptoms reported included nausea, vomiting, diarrhea, abdominal discomfort, heartburn and dizziness. Over 50% of the reported symptoms occurred during the first 2 weeks of the study and the rest were reported intermittently afterwards until the end of the study. Forty-five study participants (approximately 56% females and 44% males) reported at least one of these symptoms across all the three groups – 17 (37.8%) from HD, 13 (28.9%) from LD and 15 (33.3%) from the PL groups. None of these effects appeared to be dose-dependent or NS-related, except for the episodes of nausea. A majority of these symptoms were reported by between one and three of the subjects in any of the groups, except for vomiting, diarrhea, heartburn and dizziness, which were reported by more than three people but in no particular dose-dependent trend. For instance, a single 44-year-old female subject in the HD group was responsible for 20 of the 28 times dizziness was recorded (Table III). Heartburn incidences also appear to be high in the LD group, but only two subjects reported the effect – one subject reporting 18 of the 22 times recorded. Most of the symptoms were graded as ''mild,'' a few of them ''moderate,'' and none of them were ''severe'' incidences. Over 99% of the time, participants reported no adverse health consequences throughout the study.

Table II. Participant compliance and completion of treatment regimen.

	Treatment group			
	High dose	Low dose	Placebo	Overall
Participants				
Started	60	59	58	177
Completed (3 months)	54	53	55	162
Completion (%)	90.0%	89.8%	94.8%	91.5%
Treatment regimen				
Times capsule taken	14 847	14 697	15 035	44 579
Times capsule missed	390	543	220	1153
Total reported	15 237	15 240	15 255	45 732
Adherence (%)	97.4	96.4	98.6	97.5

In the hematological analysis, there were no significant, dose-dependent effects in any of the parameters among the three treatment groups, either before or after the 3-month trial (data not shown). In terms of time effects, only % monocytes in the white blood cell (WBC) differential analysis showed significant reductions in: HD group at end of trial ($2.2 \pm 1.7\%$, mean $\pm$ SD) compared to the baseline value ($3.2 \pm 2.1\%$) ($P < 0.05$); PL control group ($2.5 \pm 2.1\%$) at end of trial compared to the baseline value ($3.4 \pm 1.7\%$) ($P < 0.01$) (Table IV). This effect was not observed in the LD group. All other parameters were unaffected. In addition, no significant differences were observed between the NS-treated (HD and LD) and placebo (PL) groups in all the parameters evaluated.

Analysis of serum biochemistry indicated isolated statistically significant differences in a few parameters as presented in Table V. Alanine aminotransferase (ALT) level significantly increased only in the PL group at the end of trial compared to the baseline value ($p < 0.05$). Total bilirubin (T-BILI) contents marginally decreased in the HD ($p < 0.01$) and PL ($p < 0.05$) groups after 3 months of NS ingestion compared to baseline levels. This effect did not occur in the LD group. Also, blood urea nitrogen (BUN) levels were significantly reduced in all the treatment groups at the end of the study compared to baseline values. Serum creatinine (CREAT) levels slightly increased in all groups at the end of trial ($p < 0.01$). Sodium levels significantly increased at end of trial in the HD and LD groups compared to baseline values. This effect did not occur in the

Table III. Health incidences reported.

Adverse event	Treatment group			Overall
	High dose	Low dose	Placebo	
Indigestion	1[a]	1	4	6
Nausea	6	4	0	10
Vomiting	4	8	2	14
Constipation	0	1	0	1
Diarrhea	21	2	13	36
Flatulence	1	10	1	12
Loss of appetite	7	3	2	12
Abdominal discomfort	10	15	8	33
Heartburn	11	22	2	35
Dizziness	28	21	32	81
Insomnia	0	1	1	2
Bloating	0	0	0	0
No side-effect	15 148 (99.42%)	15 152 (99.42%)	15 190 (99.77%)	45 490 (99.47%)
Total incidence	89 (0.58%)	88 (0.58%)	65 (0.43%)	242 (0.53%)

[a]Indicates number of times a health incidence was reported.

Table IV. Hematological analysis.

Parameter	Treatment group						Clinical reference range
	High dose		Low dose		Placebo		
	Before trial	After trial	Before trial	After trial	Before trial	After trial	
WBC (10^9 l^{-1})	5.3 ± 3.4[a]	5.6 ± 1.9	4.9 ± 1.5	5.6 ± 1.5	5.0 ± 1.4	5.5 ± 2.1	3.4–8.9
RBC (10^6 mm^{-3})	4.9 ± 0.5	5.0 ± 0.6	4.8 ± 0.5	4.9 ± 0.6	4.9 ± 0.6	5.0 ± 0.6	2.5–5.5
HEMOGL (g dl^{-1})	13.8 ± 1.7	13.9 ± 1.5	13.5 ± 1.8	13.8 ± 1.9	13.5 ± 1.5	13.8 ± 1.6	11.7–16.5
HEMATOC (%)	43.2 ± 4.9	43.2 ± 4.4	42.7 ± 4.8	43.1 ± 5.1	41.5 ± 5.6	42.9 ± 4.5	37.1–51.4
MCV (fl)	87.7 ± 6.3	87.3 ± 6.2	89.1 ± 7.2	87.6 ± 8.1	86.3 ± 7.2	86.6 ± 6.7	86–110
MCH (pg)	27.9 ± 2.7	28.1 ± 2.8	28.1 ± 3.0	28.1 ± 3.4	32.8 ± 31.0	28.0 ± 2.5	26–38
MCHC (g dl^{-1})	31.8 ± 1.4	32.2 ± 1.4	31.5 ± 1.4	31.6 ± 2.6	31.5 ± 3.6	31.2 ± 5.3	31–37
PLT (10^9 l^{-1})	226.2 ± 90.1	223.7 ± 63.6	223.6 ± 96.0	240.6 ± 75.1	229.8 ± 69.7	231.9 ± 60.9	97–356
NEUTRO (%)	34.1 ± 9.6	36.6 ± 7.5	32.2 ± 8.9	35.5 ± 10.5	34.4 ± 9.3	37.8 ± 10.1	40–75
LYMPHO (%)	49.1 ± 9.2	46.1 ± 7.9	50.1 ± 9.6	47.9 ± 10.2	48.2 ± 9.0	47.5 ± 8.4	20–45
MONO (%)	3.2 ± 2.1	$2.2 \pm 1.7^*$	2.6 ± 1.6	2.1 ± 1.6	3.4 ± 1.7	$2.5 \pm 2.1^{**}$	2–10
EOSINO (%)	13.4 ± 7.7	15.0 ± 8.8	15.0 ± 10.0	14.4 ± 9.0	15.9 ± 17.0	12.2 ± 5.7	1–6
BASO (%)	0.2 ± 0.5	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.2	0.2 ± 0.5	0.1 ± 0.4	<1

[a]Mean $\pm$ SD; $^*p < 0.05$, $^{**}p < 0.01$ significant compared to corresponding baseline values.

PL group. Potassium level in the PL group was slightly reduced at the end of trial compared to baseline ($p < 0.01$). In all these biochemical effects, no statistically significant differences were observed between the NS-treated groups and the placebo group at the end of trial. All other serum biochemical parameters evaluated were unaffected. Two physicians (one practicing clinician in Ghana and one non-practicing in the USA) validated the results of all the parameters evaluated in the study.

Discussions and conclusions

It is well documented in the extant scientific literature that AFs are ubiquitous, naturally occurring contaminants in a variety of food products and have been associated with disease and death in humans and animals. While this problem may not pose a significant threat to developed countries, AF contamination in food products remains a serious burden in the developing world where a lack of untainted food supplies and poverty present a major and persistent challenge (McAlpin et al. 2002; Shephard 2003). Avoiding consumption of AF-contaminated foods is one of the most fundamental approaches for reducing risk of aflatoxicosis in humans. However, this is not feasible for many communities in developing countries and emphasizes the need for viable intervention strategies to manage aflatoxin-contaminated diets and treat aflatoxicosis.

One approach is chemoprevention. This strategy involves the use of natural or synthetic agents to

Table V. Serum biochemistry.

Parameter	Treatment group						Clinical reference range
	High dose		Low dose		Placebo		
	Before trial	After trial	Before trial	After trial	Before trial	After trial	
ALT ($U l^{-1}$)	13.0 ± 11.0[a]	13.8 ± 8.9	12.7 ± 7.8	15.4 ± 13.5	13.7 ± 7.6	$18.9 \pm 21.0^{*}$	0–45
AST ($U l^{-1}$)	29.1 ± 10.3	28.3 ± 14.4	30.7 ± 20.2	31.3 ± 18.2	29.2 ± 12.8	31.5 ± 12.0	0–35
ALK-P ($U l^{-1}$)	183.8 ± 77.0	183.1 ± 44.2	168.0 ± 53.9	171.7 ± 52.4	161.5 ± 45.6	162.5 ± 59.7	0–270
T-BILI ($mg dl^{-1}$)	0.8 ± 0.3	$0.6 \pm 0.4^{**}$	0.7 ± 0.3	0.6 ± 0.3	0.8 ± 0.3	$0.6 \pm 0.3^{*}$	0–1
GGT ($U l^{-1}$)	31.4 ± 14.9	33.1 ± 21.1	47.3 ± 98.5	35.0 ± 27.3	26.8 ± 12.5	29.2 ± 9.7	0–50
T-PROT ($g dl^{-1}$)	8.9 ± 1.2	8.8 ± 1.3	8.8 ± 1.3	8.8 ± 1.5	8.1 ± 1.2	8.4 ± 1.3	5–8
ALBUM ($g dl^{-1}$)	4.8 ± 0.6	$4.6 \pm 0.4^{**}$	4.7 ± 0.3	4.6 ± 0.5	4.7 ± 0.8	4.6 ± 0.5	3.5–5.5
BUN ($mg dl^{-1}$)	11.8 ± 4.7	$9.9 \pm 3.0^{*}$	13.7 ± 6.1	$11.1 \pm 3.8^{*}$	12.5 ± 4.9	$10.3 \pm 3.3^{**}$	8–23
CREAT ($mg dl^{-1}$)	0.7 ± 0.2	$1.0 \pm 0.2^{**}$	0.8 ± 0.2	$1.0 \pm 0.3^{**}$	0.8 ± 0.7	$1.0 \pm 0.3^{**}$	0.6–1.6
TRIGLYC ($mg dl^{-1}$)	89.6 ± 27.5	82.8 ± 32.1	93.0 ± 40.4	80.6 ± 30.7	82.3 ± 28.1	81.7 ± 31.0	40–200
Ca ($mg dl^{-1}$)	9.5 ± 1.3	9.1 ± 0.8	9.4 ± 1.6	9.0 ± 0.7	9.0 ± 1.2	8.8 ± 0.9	8.6–10.4
Mg ($mg dl^{-1}$)	1.9 ± 0.4	1.9 ± 0.2	2.0 ± 0.4	1.9 ± 0.3	1.9 ± 0.4	1.9 ± 0.3	1.3–2.4
Na ($mmol l^{-1}$)	142.2 ± 6.6	$146.2 \pm 9.7^{*}$	141.8 ± 6.5	$146.7 \pm 7.1^{**}$	142.6 ± 6.9	145.4 ± 8.9	120–146
K ($mmol l^{-1}$)	4.6 ± 0.7	4.4 ± 0.5	4.6 ± 0.5	4.4 ± 0.5	4.7 ± 0.7	$4.4 \pm 0.5^{**}$	3.0–5.0

[a]Mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$ significant compared to corresponding baseline values.

block, retard, reverse or modulate the carcinogenic process of AFs (Gupta and DuBois 2001; Sporn and Suh 2002). Many chemopreventive agents have been studied and some exist as natural constituents in the human diet. In numerous clinical trials, researchers have demonstrated that chemopreventive chemicals, such as oltipraz, chlorophyllins and green tea-derived polyphenols, are effective against AFs in various animal models and humans (Wang et al. 1996, 1999; Qin et al. 1997; Chi et al. 1998; Dashwood et al. 1998; Kensler et al. 1999; Klaunig and Kamendulis 1999; Nakachi et al. 2000; Egner et al. 2001; Fujiki et al. 2002). Since these compounds affect cellular metabolism, their extended use in humans would require careful evaluation, including long-term effects of enzyme modulation and interferences with the uptake of essential nutrients from the diet. In another approach, our laboratory has studied the dietary inclusion of NS clay that can preferentially bind AFs in the GI tract and reduce toxin bioavailability to the blood, liver and other organs (Phillips 1999; Phillips et al. 2002).

Results of this study indicate that administration of NS capsules (1.5 and 3.0 g day^{-1}) over a 3-month period, was apparently safe, as evidenced by physical examinations, hematological and biochemical parameters. Apart from the randomized, double-blind and placebo-control design (Figure 1), considerable efforts were made to minimize potential confounding variables. For instance, analysis of PCDD/PCDF indicated that HD and LD groups received 1000 and 2000 times less dioxin in the encapsulated NS than the WHO–THI standards. This is important because PCDD/PCDF congeners can accumulate in fatty tissues and become highly toxic to humans (Startin et al. 1990; Jensen 2001).

Study participants were selected based on pre-defined inclusion criteria, but the randomization process was conducted strictly on the basis of their serum AFB_1–albumin adduct concentrations. This deliberate design feature led to the disparity in numbers of participants (per group) regarding gender and community representation. Body weight and blood pressure values were unaffected in a dose-dependent fashion. Participants' adherence to the treatment regimens was excellent (over 97%) and more than 90% of the study subjects completed the study, which is noteworthy for a 3-month study (Table II). The NS dose levels were tolerable for the participants. None of the few symptoms reported appeared to be NS-related except for nausea. However, only one person reported nausea six times and one person reported it four times in the HD and LD groups, respectively, during the 3-month trial. It is worth noting that subjects from all groups reported having increased appetite and physical strength. The authors have not attributed this unexpected benefit to any specific cause but will investigate this trend in future studies.

Hematological analysis indicated that there were no dose-dependent significant differences between the NS-treated and PL control groups at the end of trial. In the HD and PL groups, WBC differential analysis showed significant reductions of % monocytes between the baseline and end of trial. However, this effect was well within the clinical reference range and was not observed in the LD group (Table IV). All other parameters were statistically equivalent between the NS-treated groups and the placebo control. This suggests that dietary NS is unlikely to promote inflammatory processes, impair immunity, cause alterations to bone marrow or lead to an increased incidence of infectious diseases

(Goodnough et al. 1999; George-Gay and Parker 2003).

Serum biochemical analysis showed isolated statistically significant differences in a few parameters with no particular trends of association or dose-dependency (Table V). Additionally, all measured parameters were within the normal physiological ranges. Overall, the effects of these parameters lacked dose-dependency and, thus, suggest that NS exhibited no significant adverse effects on the physiological levels of these standard biochemical parameters.

This phase IIa clinical intervention trial is the first study to evaluate the safety and efficacy of NS clay for preventing dietary AFs in human subjects. Although significant changes in a few parameters were observed, the effects did not appear to be NS-related or dose-dependent and all were within the normal physiological boundaries. This evidence suggests that short-term inclusion of NS at a minimal effective dose (MED) of 0.25% (w/w) would not likely produce overt toxicity in humans. Importantly, these findings support the prospect of using NS to rescue and protect humans who are acutely exposed to high levels of dietary AFs. Additional results from this study suggest that ingestion of capsules containing an MED of NS, significantly reduce biomarkers of aflatoxin exposure in the blood and urine from study participants (Wang et al., in preparation). Analyses of micronutrients including vitamins A and E, Fe, Zn, and other physiologically important metals have also been carried out and will be published separately. Further studies are warranted to optimize the dosimetry and delivery methods for NS. Phase IIb and phase III intervention and epidemiologic studies are also needed to confirm the safety and efficacy of NS for long-term therapy and the potential inclusion in foods for humans in areas with high incidence/prevalence of HBV and at high risk for aflatoxicosis.

Acknowledgement

We would like to express our appreciation to the District Health Director, Dr. Amponsah-Achiano, the on-site physicians, technicians and other health personnel at the Ejura District Hospital for their assistance in the physical examinations and blood collections. Special thanks to the volunteers and study subjects whose participation made this study possible. This work was supported by a research grant from the United States Agency for International Development (USAID LAG-G-00-96-90013-00) through Peanut CRSP of the University of Georgia.

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