Heavy Metals and Microbial Contaminants in a Commercial Polyherbal Product in Nigeria

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ABSTRACT: The heavy metal and microbial contaminants levels were evaluated in a commercial polyherbal product against the backdrop of reports of high levels of such contaminants in similar herbal products elsewhere in Nigeria, India and China. Atomic absorption spctrophotometric technique was used for the analysis of the herbal product for the levels of heavy metal contents while the bacterial count was by the pour plate culture method and the subsequent specific identification was achieved by sub culturing and application of Cowan and Steel procedures. The result showed that, Nasara pile syrup (NPS) contained lead, nickel, cadmium, chromium, copper and manganese in excess of the WHO permissible maximum limit for heavy metals in consumable items. The total bacteria count was 2.86 x 10^7 CFU/mL and the bacteria identified included *Bacillus cerus, Bacillus spharicus, Bacillus mycoides, Bacillus lentus, Lactus bacillus* and Yeast. The bacteria load was high enough to initiate gastrointestinal infection. These results suggest that prolong consumption of the herbal product may result in heavy metal accumulation in the body and chronic poisoning. Therefore, the distribution and consumption of this herbal product should be strictly regulated.

Key words: Nasara pile syrup, heavy metals, microbial contaminants, herbal product.

INTRODUCTION

Herbal medicine is of great importance to man and his health. More than 25% of the pharmaceutical drugs in use today originate from plant sources (Sharma, 1999). Several Asian and African countries, including Nigeria have begun to encourage herbal medicine as an integral component of their public healthcare programmes (Ajagbonna and Onyeyili, 2002). Despite all the antecedent benefits attributed to plant materials and herbal medicine, a significant percentage of human and animal health problems can be traced to the consumption of plant materials either as food or medicine. The injurious element in plant materials may come from the natural component of the plant or contaminants acquired during the process of preparation (Combest *et al*., 2005).

The presence of heavy metals in herbal preparations beyond the acceptable natural concentrations is hazardous. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues being not generally biogradable (Curtis, 2006). There have been reports of herbal medicine contamination with heavy metals. The Chinese and Indian herbal products were reported to contain nephrotoxic heavy metals such as lead, mercury, cadmium and arsenic (Combest *et al*., 2005). A great percentage (75%) of Asian herbal remedies contains heavy metals exceeding recommended oral intake (Reginster, 1997). Most of Nigerian herbal remedies have also been reported to contain heavy metals (Obi 2005). Also contamination of herbal products with micro-organisms or its derived products, fungal toxins such as aflatoxins, pesticides and synthetic drugs has been reported (Felix *et al*., 2005).

Over the years, there has been a significant increase in the production, distribution, marketing and consumption of unregistered herbal products in developing countries, Nigeria inclusive. This development is not without impending health risk. A common misconception about herbalism and use of natural products is that 'natural' equals safe. Contaminants and adulterants of medicinal plants can be pharmacologically active and responsible for unexpected toxicity (Sa'ad *et al.,* 2006). Some of these clearly identifiable herbal products being marketed and consumed by the general public in Nigeria include; potency roots, operation sweep herbal powder,

Nasara pile syrup and different Chinese herbs. In the past, several workers have investigated the toxicological profiles as well as the contaminants in these commercial herbal products (Orisakwe, 2002; Yinusa *et al*., 2006).

Recently, we have observed the rampant hawking of herbal products in containers around the Sokoto metropolis especially in motor parks, public places, artisan work areas, etc and the unrestricted manners in which people consume them. These arouse our interest to select one of these commercially available herbal products and examine the levels of heavy metals and microbial contaminants.

MATERIALS AND METHODS

Herbal Product

The investigated herbal product is marketed as Nasara Pile Syrup (NPS). An unregistered product bottled in an old gin drink bottle with a well sealed lid cover. The extract is a reddish black homogenous liquid with no apparent sediments at the bottom. It is indicated as a powerful medicine against pile and several other disease conditions. The herbal medicine enjoys a wide patronage mainly because of the increase sexual arousal effect supposedly achieved, as claimed by users.

The herbal product was purchased directly from the hawkers at the Sokoto central motor park. The batch number on the label is 04 with no indication of the material content or official registration number. A sample of the herbal medicine was deposited in the Herbarium of Pharmacology Department, Usmanu Danfodiyo University, Sokoto. The test was conducted with the original form of the herbal medicine.

Yield Value

Five millilitres of the herbal syrup were placed in a beaker and evaporated slowly at 50^{o} C. The dried powder was obtained and the percentage yield calculated.

Test for Acidity/Alkalinity

Five millilitres of the herbal medicine were warned in a hot water bath and then allowed to cool. A piece of water wet litmus paper was dipped into the filtrate and colour changed on the litmus paper observed. Red litmus paper turning blue indicates alkalinity while blue litmus turning red indicates acidity.

Test for heavy metal contaminants

The level of heavy metal contaminants in Nasara pile syrup was assessed by using Atomic Absorption Spectrometre (AAS), (model 210; manufactured by Bulk Scientific, USA). About 3.0mls of the herbal syrup were placed in the sample tube of the machine. The bar electrode for the metal of interest was set in place and this emitted the same atomic lines that was absorbed by the element present in the sample. The result as indicated on the screen of the machine was recorded. The samples were assessed in triplicates and the mean result taken as the final reading.

Test for bacterial contaminants:

(a) Total bacterial count

The total bacterial count was carried out using pour plate method as described by Barrow and Feltham (1993); Cowan and Steel (1993); Cheesbrough (2001) and Eleke and Obidiugwu (2001). The sample was serially diluted by pipetting 1.0ml of the original herbal syrup into 9.0mls of sterile distilled water to obtain a dilution of 10:1. This was further diluted to obtain a 10:7 dilution. 1.0 ml of the diluted sample was pipetted into a sterile Petri-dish plate and 20.0mls of sterile nutrient Agar maintained at 40^{0}C in water bath was added and allowed to solidify. The plates were inverted and incubated at 37^{0}C for 24 hours. After incubation, plates showing 30-300 colonies were counted and reported as colony forming units per ml while those plates yielding less than thirty or greater than three hundred (30 or 300) were ignored.

(b) Identification of specific bacterial contaminants

To identify specific bacterial contaminants, colonies on a nutrient Agar (pour plate) were sub cultured on blood and MacConkey Agar and incubated aerobically at 37^{0}C for 24 hours. The grown organisms on the media were gram stained as described by Cheesbrough (2001) and preserved on nutrient Agar Slant for further identification. Further screening test as described by Barrow and Feltham (1993) (Cowan and Steel) was carried out to identify the specific bacterium. The Cowan and Steel (1993) table of identification was used to choose the type of test suitable for a particular colony.

Test for Fungi contaminants
The test was done using Sabouraud's Dextrose Agar (SDA) as the culture medium. The SDA was inoculated with sample of the herbal medicine in Borosilicate test tubes and inoculated at 35± 2^0C for seven days. The different colonies were identified microscopically by observing their characteristic spores and hyphae (Cheesbrough, 2001) or budding cells (Andrew *et al.*, 1989).

Statistical Analysis
Analysis of data obtained from this study was done using Excel Microsoft software. Analysis of Variance (ANOVA) was used for comparison of data between and within the groups. The values were expressed as mean ± standard error of mean (SEM). Differences were considered statistically significant at 5% level ($P < 0.05$).

RESULTS
Yield Value
Five millilitres of Nasara pile syrup yielded 0.04g of dry extract powder after complete evaporation.

Heavy metal contaminants
The results obtained show that, the level of lead (1.65 ± 0.13), nickel (1.48 ± 0.03), chromium (0.25 ± 0.00), cadmium (0.24 ± 0.01), manganese (1.60 ± 0.01) and copper (0.55 ± 0.00) mg/L in the analyzed sample exceed the WHO (1991) permissible maximum limits and the Anon (2003) reference values. Zinc (0.14 ± 0.00) and iron (1.80 ± 0.08) mg/L levels were comparatively low (Table 1).

Table 1: Heavy Metal contents of Nasara pile syrup

Heavy metal	Extract content (mg/L)	Reference values (mg/L)
Zn	0.14 ± 0.00	397.500
Cu	0.55 ± 0.00*	0.110
Cd	0.24 ± 0.01*	0.031
Cr	0.25 ± 0.00*	0.011
Mn	1.60 ± 0.01*	1.400
Ni	1.48 ± 0.03 *	1.345
Pb	1.65 ± 0.13*	0.005
Iron	1.80 ± 0.08	320.000
Hg	-	-
Ar	-	-

* values exceeding regular content of natural plant values courtesy of Anon, 2003
- = Not detected.

Microbial Contaminants
The total bacteria count was 2.86 x 10^7 CFU/mL. The Coliform count was less than 3/100 mL. The organisms include 5 types of bacteria and a fungus (Table 2).

DISCUSSION
The results of this study has shown that, Nasara pile syrup contains lead, nickel, cadmium, chromium, copper and manganese in excess of the WHO (1991) permissible maximum limit for heavy metals in consumable items. Prolonged consumption of NPS may result in accumulation of a large amount of these metals in the body causing chronic poisoning and ill health (Butt *et al.*, 1996).

The human cells cannot function alone but depend on certain enzymes and other compounds. Heavy metals such as aluminum, beryllium, cadmium, lead and mercury have no known biological functions but others such as arsenic, copper, iron, nickel, in small quantities, are nutritionally essential for a healthy life. However at high levels, the metals will accumulate to cause serious health problems (Akubue, 1997).

Lead induces depression of growth when accumulated in the body (Hammond and Succop, 1994). Chronic inorganic cadmium exposure causes renal accumulation of the metal and consequent nephrotoxicity (Min *et al.*, 1996), while within the liver, pathological changes occur rapidly evidenced histologically as focal necrosis, fibrosis, fatty infiltration and cirrhosis (Kayama *et al.*, 1995). Epidemiological studies revealed that skin, lungs, liver and bladder cancers were associated with arsenic exposure via inhalation or ingestion (Fevesitech *et al.*, 2006). Chromium, another metal found in excess in NPS, has been associated with incapacitating eczematous dermatitis and hepatitis (Akubue, 1997). It is known that the incidence of lung cancer is increased by up to 15 times the normal in workers exposed to chromium ores and its derivatives and, chronic intoxication from manganese may result in dermatitis and liver enlargement which later may produce a parkinsonial like effect (De Smet *et al.*, 1992).

This study has also shown the presence of some microorganisms as contaminants in NPS. Although herbs are biological substances and hence will naturally contain large numbers of a

Table 2: Test results for the Microbial contents of Nasara pile syrup

	TYPE OF TEST											
S/NO	**G/R**	**morphology**	**Cat**	**H_2S**	**Motility**	**Glucose**	**Lactose**	**Sucrose**	**Indole**	**Spores shape & position**	**Vp**	**Bact. Specie**
1	+	Rods	+	+	+	-	-	-	-	Oval central	+	*B. cerus*
2	+	Rods	+	+	-	-	-	-	-	Round terminal	-	*B. spharicus*
3	+	Rods	-	-	-	-	-	-	-	Oval central	-	*B. mycodes*
4	+	Rods	-	+	-	-	-	-	-	Oval sub-terminal	-	*B. lentus*
5	+	Rods	-	+	-	+	-	-	-	-	-	*L. Bacillus*
6	-	Oval	-	-	-	-	-	-	-	-	-	Yeast cells

+ = presence; - = absence; G/R = Gram reaction test; Morph =; Mot =; Glu =; Lact =; Suc =; Ind =; **Vp** = Vogesprauskaur Test

variety of microorganisms (Kerry, 2003), the bacterial load discovered in NPS is sufficient to initiate an infective process especially in the gastrointestinal tract. Surveys of crude medicinal herbs both in Nigeria and abroad have found high levels of total aerobic organisms, enterobacteriaceae and coliform organisms, yeast and moulds. (Kneifel, *et al*., 2002; Eleke and Obidiugwu, 2001). Heavy metals and microbes contaminants were reported in roots of *Valeriana officinalis* in the United Kingdom (Richard, 2007). Of serious concerns than the mere presence of the microorganisms in herbal products are mycotoxins and toxic metabolites such as aflatoxin produced by fungi which are carcinogenic and teratogenic (Hirokoto *et al*., 1978; Martins *et al.*, 2001).

Overall, this study has shown that, Nasara pile syrup (NPS) contains excessive levels of copper, cadmium, chromium, manganese, nickel and lead which after a prolonged consumption may accumulate in the body and predispose the consumer to various health problems. The level of microbial contaminants present is high enough to initiate gastrointestinal infection. Therefore, adequate measures should be taken by the Drug Regulatory Agency in Nigeria to discourage the marketing of herbal products that have not been evaluated and licensed. The Indian government in response to the reports of metal contamination in herbal/ ayurvedic medicine issued a notification making it mandatory for the testing of levels of heavy metals in herbal products meant for exports as from January 2006. So, the Nigerian government through its drug Agency is recommended to do the same.

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