Heavy metal content of commonly consumed herbal bitters in Ilorin, Nigeria

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Abstract

Background: The use of herbal medicines has increased in recent years and has gained much attention in the health sectors, scientific community and the public alike. The safety and quality of these products become questionable even when the efficacy and potency may be guaranteed.

Objective: The presence of heavy metals was determined.

Methods: Two batches of five herbal bitters were purchased from different parts of Ilorin. Samples were prepared from these batches and analyzed for the presence of Cadmium (Cd), Iron (Fe) and Lead (Pb) using Atomic Absorption Spectrophotometer. Their concentrations were compared with WHO permissible limits. The variations in the concentration of Cd, Fe, and Pb in the two batches of samples were also determined at $P < 0.05$.

Results: The value of Cd ranged from 0.003-0.3mg/L, Pb 0.000-0.067mg/L and Fe 0.083-0.27mg/L. This study revealed that majority of the samples contained Cd and Pb in concentrations significantly lower than the permissible limits. However, the two batches of herbal bitters contained Fe with concentrations significantly higher than the official permissible limit of 0.1mg/L.

Conclusion: The results obtained from this study showed that Iron was present in all the samples with some concentrations significantly higher than the WHO permissible limit. Lead and cadmium were present in some of the samples with concentrations below the WHO permissible limit.

Key words: Herbal bitters, lead, cadmium, iron, atomic absorption Spectrophotometer .

La teneur en métaux lourds des amers à base de plantes consommés couramment à Ilorin, au Nigeria

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RESUME

Contexte: L'utilisation de plantes médicinales a augmenté ces dernières années et a reçu beaucoup d'attention dans les secteurs de la santé, la communauté scientifique et le public. La sécurité et la qualité de ces produits deviennent douteuses même lorsque l'efficacité et la puissance peuvent être garanties.

Objectif: La présence de métaux lourds a été déterminée.

Méthodes: Deux lots de cinq amers à base de plantes ont été achetés à des endroits divers d'Ilorin. Des échantillons ont été préparés à partir de ces lots et analysés pour déterminer la présence de cadmium (Cd), de fer (Fe) et de plomb (Pb) au moyen d'un spectrophotomètre d'absorption atomique. Leurs concentrations ont été comparées avec les limites autorisées par l'OMS. Les variations de la concentration en Cd, Fe et Pb dans les deux lots d'échantillons ont également été déterminées à $P < 0,05$.

Résultats: La valeur de Cd variait de 0,003-0,3 mg/L, Pb 0,000-0,067mg/L et de Fe 0,083-0,27mg/L. Cette étude a révélé que la majorité des échantillons contiennent du Cd et du Pb dans des concentrations nettement inférieures aux limites autorisées. Cependant, les deux lots d'amers à base de plantes contenaient du Fe avec des concentrations significativement plus élevées que la limite officielle autorisée de 0,1 mg/L.

Conclusion: Les résultats obtenus à partir de cette étude ont montré que le fer était présent dans tous les échantillons avec certaines concentrations significativement supérieures à la limite permise par l'OMS. Le plomb et le cadmium étaient présents dans certains des échantillons dont la concentration était inférieure à la limite autorisée par l'OMS.

Mots clés: Amers à base de plantes, Plomb, Cadmium, Fer, Spectrophotomètre à absorption atomique

INTRODUCTION

The use of homeopathic and herbal medicines has increased in recent years (Chikezie *et al*, 2015)[1]. This has probably arisen as a result of a number of factors including; disillusionment with conventional drugs, growing confidence in complementary medicine, and a belief that the products are safe, often on the grounds that 'natural' equates to safe.[2] Herbal medicine and traditional treatment are the main source of health care and sometimes the only source of care for millions of people in developing countries (WHO, 2013).[3] A survey carried out in Lagos, Nigeria also reported that majority of the respondents use herbal medicines.[4] However, the challenges associated with the use of these medicinal plants and herbal products obtained from them include safety and quality even when efficacy and potency are ascertained.

The presence of heavy metals in the body can be detrimental to health particularly at concentrations above tolerance level. Most are considered toxic to living organism due to their accumulative tendency in tissues resulting in various disorders.[5] It has been reported that the presence of heavy metals in either orthodox or herbal drugs often compound the original ailments for which they were administered with resultant increased morbidity for the patient.[6] There are various sources of contamination that renders the safety and quality of medicine questionable and notable amongst them is that from heavy metals. Contamination of traditional medicines by heavy metals is of major concern because of the toxicity, persistence and bio-accumulative nature of these metals.[7] Examples of heavy metals include Arsenic (As), Lead (Pb), Mercury (Hg), Copper (Cu), Iron (Fe), Cadmium (Cd) and Zinc (Zn). Three main sources have been proposed to be responsible for heavy metal contamination of medicinal herbal products,[8] viz; contamination during the process of cultivating the medicinal plant itself, [9,10] unintentional cross contamination during processing,[11] and the deliberate addition of heavy metals as therapeutic agents.[12,13] The WHO has formulated guidelines for quality assurance and control of herbal medicine but most traditional practitioners lack this information.[14] This sometimes results in medicinal plants and products derived from them being contaminated with various types and concentration of heavy metals.

Some reported works established the fact that there are both heavy metal pollution of the environment and contamination of herbal products in Nigeria and the world at large.[15] In a study conducted in Nigeria to determine the cadmium (Cd) concentrations of herbal drugs used as antimalarial and that of chloroquine syrup, it was revealed that Cadmium was detected in 100 % of the herbal products and 75 % of the chloroquine syrups.[16] The concentration of Cd in all the herbal products exceeded the WHO limit, while the concentration in all the chloroquine syrups were within the British Pharmacopeia (BP) 2002 limit.[16] Another study reported the concentration of Fe ranged from 65.68-1652.89 µg/g in different products of herbal medicine purchased from various places in Karachi city of Pakistan.[17]

A lot of cases of poisoning from heavy metal such as cadmium, lead, iron, mercury and manganese remain underreported while some are undocumented due to poor record keeping in the developing nations.[2, 18] Therefore, the need for more studies investigating the safety and quality of the herbal products becomes imperative.

This research focused on various herbal bitters acclaimed to be used for a variety of health conditions within Ilorin Metropolis of Nigeria. The safety and quality of two different manufacturing batches of herbal bitters, in terms of their level of contamination by heavy metals were ascertained. The heavy metals selected for this study include two biologically non-essential elements: Lead (Pb), Cadmium (Cd), and one biologically essential element: Iron (Fe) which are some of the heavy metals implicated to be toxic in human.

METHODS

Collection of samples

Five registered Pharmacies were selected using convenience sampling method from the three local Government Areas that make up Ilorin metropolis, viz: Ilorin South, West and East local Government Areas. Herbal bitters were purchased from these registered pharmacies. Five different herbal bitters having two batches were selected for this study. Herbal bitters with only a batch available at the time of study were excluded. They were coded A_1, B_1, C_1, D_1 and E_1 (Batch 1) and A_2, B_2, C_2, D_2, and E_2 (Batch 2). The herbal bitters were in their original packages as supplied by the manufacturers and examined carefully for the manufacturing dates, expiry dates, NAFDAC registration numbers and their manufacturing batch numbers.

Equipment and reagents

Buck Accusys model 211 Atomic Absorption Spectrophotometer (AAS) equipped with corresponding hollow cathode lamp (Lead, Cadmium and Iron) at the time of analysis, De-ionized water, H_2SO_4, HNO_3, NO_2. All experiments were performed using analytical grade of the reagents.

Preparation of calibration curve

De-ionized water was used in preparing solutions. Stock solutions of each metal Pb, Fe (undifferenciated), and Cd were prepared by dissolving each solid metal sample (1.0 g) in 10 mL 1:1 nitric acid solution. The solution was transferred into a 1000 mL volumetric flask and made up to mark with de-ionized water.

Standard solutions were prepared from each metal stock solution of 1000 mg/L.[5] A 100 mL quantity of the standard solution of each metal was adjusted to pH of 2.5 by adding 1M nitric acid. Each standard solution and blank was transferred into an individual 250 mL separating funnel. 1 mL ammonium pyrrolidine dithiocarbamate was added followed by the addition of 10 mL methyl isobutyl ketone and the solution was shaken vigorously for 2 minutes and allowed to settle. The aqueous layer was discarded; while the organic layer was then aspirated directly into the flame and the absorbance was recorded using an Atomic Absorption Spectrophotometer equipped each with hollow cathode lamp, at 283.2 nm, 248.3 nm and 228.8 nm wavelengths for Pb, Fe and Cd respectively. The nebulizer, atomizer and burner were flushed each time with de-ionized water after each sample was aspirated before the next. The stability of the equipment was checked at intervals by introducing the highest working standard solution and the blank.[5]

Sample pre-treatment

A 10 mL quantity of each herbal bitter sample was measured into a digestion flask and digested with 10 mL of tri-acid mixture (HNO_2:$HClO_4$:H_2SO_4) in the ratio 25:4:2 i.e. 8.1 mL HNO_3, 1.3 mL $HclO_4$, 0.6 mL H_2SO_4 on a hot plate under a fume cupboard at 100 °C until dense white fumes appeared. The flask was allowed to cool, a quantity of de-ionized water was added and digested until a clear solution was obtained. The solution was cooled and filtered into a 100 mL volumetric flask and made up to mark with de-ionized water.[19] This pre-treatment was done in triplicate for each sample and a total of thirty (30) digests were obtained.

Sample analysis

The digested samples were analyzed using BUCK ACCUSYS 211 Atomic Absorption Spectrophotometer equipped with hollow cathode lamp, at 283.2 nm, 248.3 nm and 228.8 nm wavelengths for Pb, Fe and Cd respectively. The absorbance for each metal (Pb, Fe, and Cd) in each digested sample was recorded and concentration of metal determined from the calibration curve.

Statistical analysis

The results were expressed as the mean ± S.E.M. Data was analyzed using GraphPad Prism (Version 7). Statistical analysis was carried out using Student's t-test to compare heavy metal content in the herbal bitters with the WHO permissible limit. The statistical significance was taken at $P < 0.05$.

RESULTS

Heavy metals (Pb, Fe and Cd) were present in samples obtained from the herbal mixtures used in this study. The quantitative analysis showed that Lead and cadmium were absent in most of the samples (Table 1) except in A_2, E_1 and E_2. Iron was present at different concentrations in both batches of all samples as presented in Tables 2 and 3.

Table 1: Concentration of Pb, Fe and Cd in mg/L in batch 1 samples

Sample code	Pb mg/L	Fe mg/L	Cd mg/L
A_1	0.000±0.000	0.107±0.015	0.000±0.000
B_1	0.000±0.000	0.083±0.007	0.000±0.000
C_1	0.000±0.000	0.270±0.086	0.000±0.000
D_1	0.000±0.000	0.127±0.003	0.000±0.000
E_1	0.000±0.000	0.140±0.035	0.003±0.003

Values: means ± S.E.M (n=3)

Table 2: Concentration of Pb, Fe, and Cd in mg/L in batch 2 samples

Sample code	Pb mg/L	Fe mg/L	Cd mg/L
A_2	0.067±0.033	0.140±0.015	0.000±0.000
B_2	0.000±0.000	0.087±0.012	0.000±0.000
C_2	0.000±0.000	0.167±0.012	0.000±0.000
D_2	0.000±0.000	0.210±0.006	0.000±0.000
E_2	0.000±0.000	0.120±0.006	0.003±0.003

Values: means ± S.E.M (n=3)

Concentration of Pb, Fe and Cd in herbal bitters compared with permissible limits

The concentration of heavy metals (Pb, Fe and Cd) in the samples were compared with permissible limit of 10 mg/L, 0.3 mg/L and 0.1 mg/L for Pb, Cd and Fe respectively.[27] The concentration of Iron was significantly (P=0.01) higher than the permissible limits in samples C_1, C_2, D_1 and D_2 while concentration of Lead in sample A_2 was lower than the permissible limit as shown in Tables 3 and 4.

Table 3: Comparison of concentrations of Pb, Fe and Cd with permissible limits for batch 1 samples

Sample code	Concentrations (mg/L) / Permissible limits (mg/L)		
	Pb	Fe	Cd
A_1	0.067±0.000/10.000	0.107±0.015/0.100	0.000±0.000/0.300
B_1	0.000±0.000/10.000	0.083±0.007/0.100	0.000±0.000/0.300
C_1	0.000±0.000/10.000	0.27±0.086*/0.100	0.000 ±0.000/0.300
D_1	0.000±0.000/10.000	0.127±0.003*/0.100	0.000 ±0.000/0.300
E_1	0.000±0.000/10.000	0.140±0.035/0.100	0.003±0.003/0.300

Values: means ± S.E.M (n=3). *P<0.05 vs. Permissible limits; Student's t-test

Table 4: Comparison of concentrations of Pb, Fe and Cd with permissible limits for batch 2 samples

Sample code	Concentrations/ Permisible Limits/ SEM (mg/L)		
	Pb	Fe	Cd
A_2	0.067±0.000/10.000	0.140± 0.015/0.10	0.000±0.000/0.300
B_2	0.000±0.000/10.000	0.087 ±0.012/0.10	0.000±0.000/0.300
C_2	0.000±0.000/10.000	0.167±0.012*/0.10	0.000±0.000/0.300
D_2	0.000±0.000/10.000	0.210 ±0.006*/0.10	0.000±0.000/0.300
E_2	0.000±0.000/10.000	0.120 ±0.006/0.10	0.003±0.003/0.300

Values: means ± S.E.M (n=3). *P<0.05 vs. Permissible limits; Student's t-test

The concentration of iron in batch 1 and 2 samples were compared as shown in Figure 1

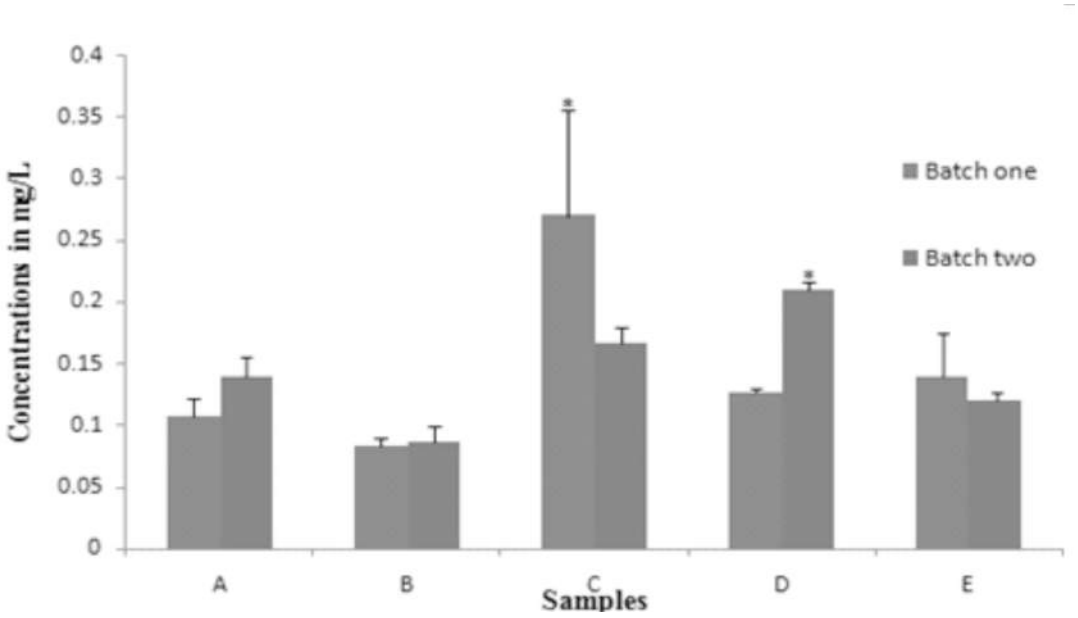

Figure 1: Concentrations of iron in samples from two (2) batches of herbal bitters n=5. *P<0.05

DISCUSSION

Heavy metals (cadmium, iron and lead) were present in the samples of herbal bitters selected for this study. The result of the quantitative test for cadmium revealed that two samples from two different batches of herbal bitters representing 20 % of the samples contained cadmium at a concentration of 0.003 mg/L. This concentration was lower than the permissible limit of 0.3 mg/L recommended by WHO.[20] Consumers of these herbal products may not be at risk of cadmium toxicity with manifestation of nephrotoxicity, aminoaciduria, glycosuria and tubular necrosis [21, 22, 23] since its concentration therein was very low. A study conducted in Zaria, Northern Nigeria showed that cadmium (Cd) concentration exceeded the WHO limit by 100 % in all the herbal drugs used as antimalarial.[16]

In the present study, 10 % of samples from the herbal bitters analyzed were found to contain lead. The concentration of lead was between 0.000 and 0.067 ± 0.033 which was lower than the WHO permissible limit of 10 mg/L. The presence of this amount of lead in the herbal bitters may not pose health threat, although human users should be advised not to use these products over a long period of time. This result is similar to the findings obtained from a study carried out in Ghana on hazardous concentration of lead in traditionally used herbal drugs.[24]

In addition, the results obtained from this study showed

that all the samples of herbal bitters contained iron with its concentration varying between 0.083 mg/L and 0.27 mg/L. It is important to note that four (4) samples from the herbal bitters analyzed contained iron in concentrations significantly higher than the 0.1 mg/L permissible limit recommended by WHO, 2006. There was also a significant difference in the iron concentrations between samples of two different batches of the herbal bitters. Another study reported the concentration of iron to be between 0.0001 and 1.12 ppm in different herbal medicine purchased from various places in Karachi city of Pakistan[17] and KwaZulu-Natal Province, South Africa.[24]

Previous studies have shown that when consumers take above the permissible safe limit of iron, either from nutritional sources, or from orthodox or herbal drugs, untoward reactions may occur.[25] Also, cases of iron ingestion leading to toxicity or poisoning have been reported in some studies.[23] Iron toxicity may cause gastrointestinal effects such as vomiting, diarrhea, melena, and abdominal pain which may occur and progress to life threatening hemorrhagic gastritis, perforation, and peritonitis.[26] Such cases of acute gastroenteritis may contribute to early cardiovascular toxicity through fluid and blood loss which manifests as decreased cardiac output, cardiac arrest and shock.[27] Also, metabolic acidosis and CNS manifestations such as depressed sensorium, ranging from mild obtundation to profound coma are commonly seen with severe iron overdose.[28,29]

Iron preparations are beneficial in the management of iron deficiency anaemia and a deliberate addition by the manufacturer of the herbal bitters cannot be overruled. The high concentrations of Fe found in the two batches may be due to factors such as contamination during the process of cultivating and/or harvesting the medicinal plants, unintentional cross contamination during production, and deliberate addition of heavy metals as therapeutic agents. Critical evaluation is recommended to be carried out to detect the source(s) of iron contamination in the samples of herbal bitters analyzed. Public awareness and or enlightenment about the adverse effects of iron overload in herbal bitters may be extremely needful.

The three heavy metals (Cd, Pb and Fe) chosen for analysis was a limitation for this study, since the AAS used was calibrated for these metals only. In addition, only five herbal bitters had two different batches available in the pharmacies while others had just one batch at the time of sample collection.

CONCLUSION

The results obtained from this study showed that iron was present in all the samples with some concentrations significantly higher than the WHO permissible limit. Lead and cadmium were present in some of the samples with concentration below the WHO permissible limit. This study recommends that regulatory bodies should ensure herbal products are free from heavy metal contaminants and safe for human consumption.

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