academicJournals

Vol. 9(3), pp. 23-28, March 2017
DOI: 10.5897/JTEHS2016.0378
Article Number: E7F98D563020
ISSN 2006-9820

http://www.academicjournals.org/JTEHS

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Herbal teas heavy metal evaluation with renal function assessment in regular consumers in Benin

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Received 27 December, 2016; Accepted 25 January, 2017

Herbal teas are openly sold in markets and main streets of big cities like Cotonou in Benin. Most people treat themselves at low cost with wide variety of herbal plants with proven therapeutic properties. The purpose of this research was to evaluate the content of heavy metals in herbal teas sold daily in Cotonou. In addition, we would evaluate if this heavy metal content of herbal teas could affect the renal function of regular consumers. Therefore, herbal teas samples were collected from selected sellers at well-known places. Samples' analyses were done by reverse anodic stripping voltammetry with the Metalyser HM 3000 coupled to PC 101 NT pump. Biological markers of kidney failure in blood and urine of regular consumers were also assessed. Renal creatinine clearance and albuminuria were measured, the ratio of micro-albuminuria/creatininuria was calculated, and then red and white blood cells were counted. The results indicate that 7.69 and 30.77% of the herbal teas samples displayed an abnormal high concentration in cadmium (Cd) and lead (Pb) concentration, respectively. Statistical significant difference was found between the analyzed herbal tea samples in regard to Cd and Pb content ($P < 0.01$). No obvious biological sign of severe kidney damage has been noted among regular consumers using their blood and urine samples. Only 8.60% of them had clearances between 60 and 89 ml/min/1.73 m^2 with mild kidney failure. This study indicates that some herbal teas contain toxic chemicals such as Cd and Pb over recommended limits of 3 and 10 µg/L, respectively. The regular consumption of these herbal teas could be health threatening for the population.

Key words: Herbal teas, heavy metals, environmental pollution, human renal function, anodic stripping voltammetry.

INTRODUCTION

In Benin, traditional cure such as herbal teas are openly sold in markets and main streets of main cities like Cotonou. People consumed herbal teas for medical purposes and keeping their health. They treat themselves at lower cost from a large biodiversity of medicinal plants with proven therapeutic properties. However, medicinal

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plants can be easily contaminated by heavy metals during cultivation or later during the processing stage (Jabeen et al., 2010). Literature has described some toxic heavy metals in herbal teas (Samali et al., 2012). Along with other pollutants, heavy metals can be released into the environment through industrial activities, household wastes, engine exhaust, pesticides and fertilizers used in agriculture (Järup, 2003). Generally, the concentrations of heavy metals in herbal teas changed according to the types of herbal teas and geological conditions. Medicinal plants often used in Cotonou under maceration, decoction or infusion forms can be contaminated in their biosphere or during processing (Marcos et al., 1998). Lead (Pb) and cadmium (Cd) are trace metals which are not essential for either humans or plants, while could easily induce toxic effects in humans at low concentrations. For crops, the lowest range of Cd which can cause yield reduction is 5 to 30 $\mu g/kg^{-1}$, while its maximum allowable concentration in edible plants is as low as 1 $\mu g\ kg^{-1}$ (Esetlili et al., 2014). Therefore, herbal tea safety has piqued great interest because contaminants threaten the life and health of humans, animals and the environment, leading to economic losses (Baranowska et al., 2002; Friberg and Lene, 1986). The genetic and epigenetic effects of dietary heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As) and selenium (Se) in the human body are associated with an increased risk of different cancers (Hunt, 2003). Thus, the harmful effects of medicinal plants can be linked to an indirect contamination of those plants by heavy metals and toxic or radioactive agrochemical residues (Zeggwagh et al., 2013). Prolonged consumption of heavy metals from food can lead to their accumulation in the kidney and liver, causing disruption of numerous biochemical processes and potentially causing cardiovascular, nervous, kidney and bone diseases (Jabeen et al., 2010). In order to protect human health from Cd and lead intoxication, a limit was set by the world health organization (WHO) to 3 µg/L for Cd and 10 µg/L for Pb (World Health Organization, 2011a, b). It has been reported that the average quantities of lead and cadmium measured in *Senna rotundifolia* L. extracts in Benin were well above recommended limits of the WHO (Montcho et al., 2014). In our knowledge, until now, no scientific work in Benin has tackled the possible high content of heavy metal in herbal teas commonly called “adômansin”. These herbal teas are frequently drunk by a large portion of the population especially in the population with poor and middle income. According to WHO, toxic heavy metals have to be controlled in herbal plants in order to assure their safety (World Health Organization, 1998; Hussain et al., 2011). Therefore, controlling the heavy metal concentrations in teas and biological fluids of regular consumers should be done to ensure the safety of herbal teas. The concentrations of heavy metals have not been reported in herbal teas available in Cotonou, Benin. This study was intended to determine the concentration of Cd and Pb in selected herbal teas daily sold in Cotonou and assess some of the biological markers of kidney failure among regular consumers. The data obtained could be used for the quality control process to ensure the purity and safety of medicinal plants used for herbal teas in Benin.

MATERIALS AND METHODS

Instrumentations

Anodic stripping voltammetric (ASV) determination of Cd and Pb were performed with trace metal analyzer (Metalyser HM 3000) with three-electrode system coupled to PC 101 NT pump.

Sample collection and preparation

Herbal tea selling was practiced along many streets in Cotonou. Thirteen sellers were randomly selected in well-known places and herbal tea samples were purchased from them. Each sample is a mixture of several medicinal plants. The content of these mixture changes from one seller to another. A volume of 10 ml of herbal tea samples were collected in volumetric flask for the determination of Cd and Pb and analyzed within the next 4 h.

Preparation of standard solution

Reference standards of Cd and Pb were purchased from Inorganic Ventures, Inc. Lakewood, NJ and used for the preparation of aqueous standard solutions of 1.000 g/L. Mixed working solution (1.000 mg/L) of Cd and Pb was prepared by appropriate dilution for the voltammetric analysis from stock solution of 1.000 g/L.

Dosage of cadmium and lead

Ultra-pure water (10 ml) and 1 ml of acetate buffer (pH 4.6) were taken in polarographic vessel, well mixed, and the voltammogram of the blank was recorded. Volume of 1 ml of herbal tea sample was added into the polarographic vessel and then voltammogram of the sample solution was recorded under the same conditions. After the sample voltammogram was recorded, 0.2 ml of 1 mg/L mixed standard of Cd and Pb was added and then voltammogram of the standard was recorded. The procedure was repeated three times and the current was measured. The concentrations’ values of Cd and Pb were directly displayed on the screen consecutively with the voltammogram. The limit of detection is 0.5 µg/L for both analytes.

Recruiting regular herbal tea consumers

A survey with the herbal tea sellers was conducted to identify regular consumers. Only clients who were faithful to the sellers, aged over 18 years, and who have been drinking herbal tea for more than three months, were included in the study. Consumers with medical background of diabetes, high blood pressure, obesity, chronic renal failure, urinary infection and other chronic pathology, were excluded. Subjects who did not give their informed consent were not included.

Renal function assessment of regular herbal teas consumers

Thirty five regular consumers were received at the office of the

Table 1. Cadmium and lead content of the herbal teas samples.

Herbal tea sample	Cadmium [Mean±SD] (µg/L)	Lead [Mean±SD] (µg/L)
T1	0.853 ± 0.31	0.942 ± 0.25
T2	0.783 ± 0.45	4.073 ± 1.32
T3	1.977 ± 0.42	20.798 ± 2.35 *
T4	1.103 ± 0.49	17.519 ± 2.99 *
T5	0.864 ± 0.22	1.029 ± 0.32
T6	0.833 ± 0.25	1.036 ± 0.18
T7	1.389 ± 0.76	10.675 ± 2.25 *
T8	1.192 ± 0.71	10.120 ± 2.35 *
T9	0.945 ± 0.10	0.440 ± 2.21
T10	1.050 ± 0.22	0.967 ± 0.41
T11	0.937 ± 0.43	0.896 ± 0.22
T12	5.536 ± 1.63 *	8.770 ± 3.03
T13	1.031 ± 0.24	0.892 ± 0.11
Average	1.423 ± 0.48	6.012 ± 2.01

*High content of Cd or Pb.

National Laboratory of Narcotics and Toxicology (LNST) in Cotonou. The study was approved by the local ethical committee, and all volunteers gave their written consent. Blood (4 ml) was drawn from each volunteer into a dry test tube. The samples were centrifuged and serums were harvested for the creatinine level determination using JAFFE method. Urine samples were also collected from each subject. Before collecting urines, every participant was asked to empty his bladder. Then, urine of the following 3 h was collected into plastic bottles and the following analysis was performed: creatininuria, albuminuria, red and white blood cell count, and determination of the ratio micro-albuminuria/creatininuria.

Statistical analysis

Analysis of variance (ANOVA) was realized to verify the existence of significant difference between thirteen herbal teas studied for Cd and Pb contents. Data were processed with R software R 3.3.2 (R Development Core Team, 2016). At first, normality of data and equality of variances were verified, respectively with Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene (Zar, 1999) tests. Student test, Newman and Keuls tests were used for statistical analysis and comparison, and outcomes were represented by barplot with errors bars.

RESULTS

Heavy metals concentrations

The concentration of Cd and Pb contained in each herbal tea sample are recorded in Table 1. The normal values authorized for these two metals are respectively less than 3 and 10 µg/L. From our study, 7.69% of the herbal teas samples had a high concentration in cd, while 30.77% of those samples indicated high lead content. The statistical analysis showed statistical significant different between the analyzed samples in regard to Cd and Pb content. The results of Cd content showed that three groups were statistically different ($P < 0.01$), the first is constituted by T12 only, second is composed of T3 and third is constituted by T1, T10, T11, T13, T2, T4, T5, T6, T7, T8 and T9 (Figure 1). Meanwhile, the results of Pb content showed eight groups were statistically different with $P < 0.01$ (Figure 2).

Periods of herbal teas consumption

The volunteers enrolled in this study have several years' experience in drinking these herbal teas. The majority of the volunteers (43%) have been drinking these herbal teas for five to ten years, while 40% have been drinking over ten years.

Assessment of renal function

Socio-demographic characteristics

The socio-demographic data of the 35 volunteers were reviewed in this study. The median age of the volunteers was 42 (range 21 to 66) years, while the mean age was 40±12 years (M±SD). The socio-demographics of the volunteers are shown in Table 2.

Clearance of creatinine

The study found that 8.57% of the volunteers had a glomerular filtration rate between 60 and 89 ml/min/1.73 m^2 indicating that they were suffering from mild renal failure. Table 3 displays renal clearance values according to the Modification of Diet in Renal Disease (MDRD) method.

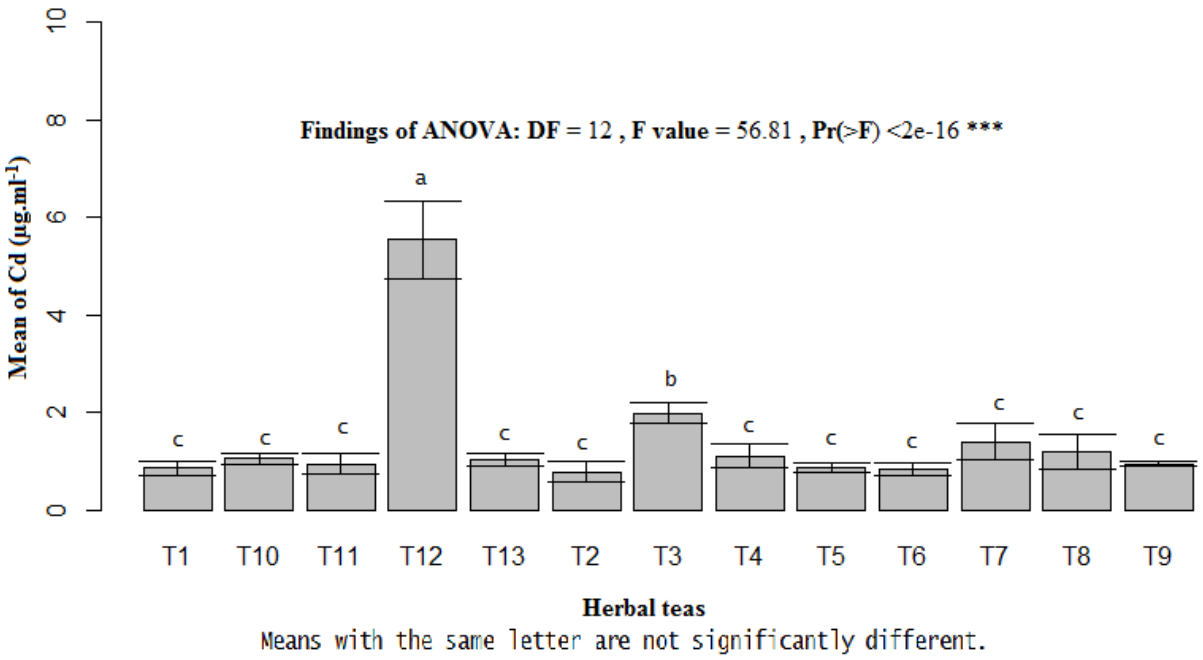

Figure 1. Comparison of cadmium content in herbal teas.

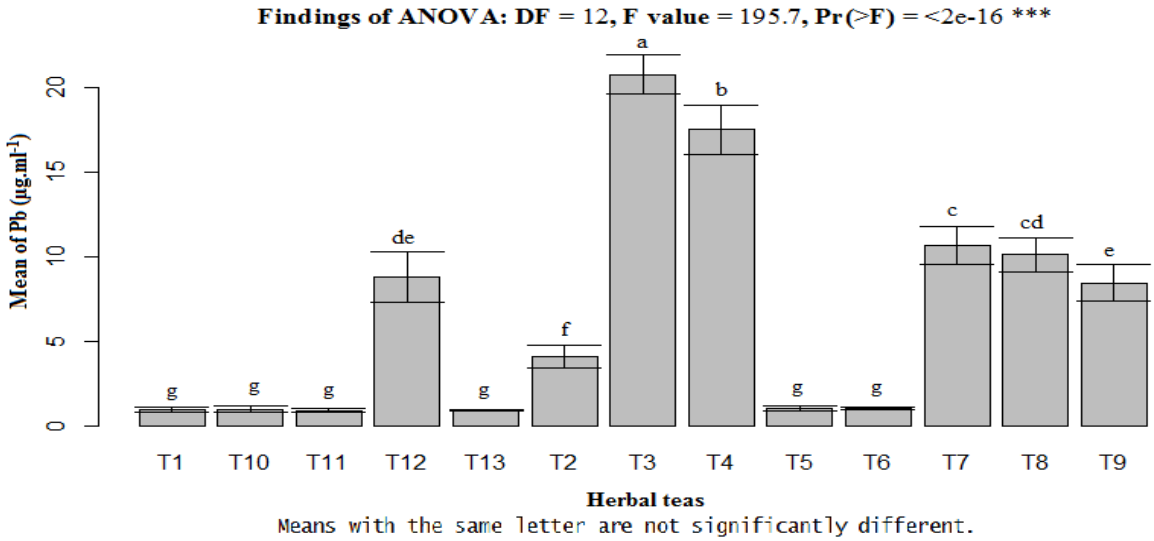

Figure 2. Comparison of lead content in herbal teas.

Micro-albuminuria/creatininuria ratio

All data were within the normal range values for micro-albuminuria/creatininuria ratio for each volunteer as shown in Table 4.

Number of red and white blood cells in urine

All participants had the red and white blood cells count inferior to 5000. No abnormality was found. Table 5 displays the numbers characterizing each volunteer.

DISCUSSION

Identification of heavy metals

In the recent years, human activities, such as industry

Table 2. Socio-demographic data of regular herbal teas consumers.

Socio-demographic data	Results	
Sex of the consumers	94% (n=33) of the regular herbal teas consumers enrolled were male	
Age of the consumers	Minimum	21
	Maximum	66
	Average	40.31
	Standard deviation	11.87
	Median	42
	Interquartile range	28-46
Place of residence of the consumers	51.43% (n=18) of regular consumers mainly come from the suburban area (51.43%)	
Professions of the consumers	40% of the participants were motor bike taxi drivers commonly called "*zémidjans".* Craftsmen occupy the second position (31.5%). No other profession reaches the percentage of 3% of the study population.	

Table 3. Renal creatinine clearances.

Clearance (ml/min/1.73 m^2)	Interpretations	Number of consumers (n=35)
≥90	No renal failure	32
60 – 89	Mild renal failure	03
30 – 59	Moderate chronic renal failure	00
15 – 29	Severe chronic renal failure	00
<15	Terminal chronic renal failure	00

Table 4. Micro-albuminuria/creatininuria ratio of the consumers.

Ratio (mg/g)	Number (n=35)
<30	35
≥30	00

Table 5. Red and white blood cells count in the urines of consumers.

Number of cells per minute	Number (n = 35)
Red blood cells <5000/min	35
Red blood cells ≥5000/min	00
White blood cells <5000/min	35
White blood cells ≥5000/min	00

and agriculture, promote heavy metal release into the environment. Thus, the analytical determination of metals in medicinal plants has become a part of quality control in order to establish their purity, safety and efficacy (Baranowska et al., 2002). In this study, 7.69% of the herbal teas samples showed a high Cd concentration with an average of 5.84 µg/L and 30.77% of the same samples showed a high Pb concentration. According to Montcho et al. (2014), heavy metals persist in the ground because they are not chemically or biologically deteriorated. Heavy metals could end up in herbal teas from waste products containing manufactured items such as batteries, electronic wastes which are thrown in the environment, and then pollute the rain water used by medicinal plants for their growth. The heavy metals are stocked in the leaves, roots, and barks of those plants. These heavy metals can also accumulate in clay or organic matter by complex connections or under ionic form in solution (Montcho et al., 2014). They can also form inorganic compounds or get fixed to the surface of particles by adsorption. According to Adam et al. (2010), norms about heavy metals, Cd and Pb concentrations of drinkable water should be respectively inferior to 3 and 10 µg/L.

In Montcho et al. (2014) study, samples of *Chamaecrista rotundifolia* (Pers.) (Greene.) collected on Dantokpa's, Vossa's and Godomey's markets in Benin had respectively high Pb concentrations of 2733, 1825, and 1902 ppb. The difference in Cd and Pb concentrations between our study and that of Montcho et al.

(2014), could be explained by the fact that this study quantified heavy metals in the entire decoction (drinkable solution) made of several medicinal plants, while Montcho et al. (2014) worked directly with *C. rotundifolia* (Pers.) (Greene.) plant. Heavy metals can seriously damage health. According to Stengel (1996), Cd causes proximal renal tubulopathy in the human and the main sign of the disease is a proteinuria of small molecular weight often associated to Fanconi' syndrome. The frequency of renal stones is increased in workers exposed to Cd. The tubular proteinuria observed is irreversible and is accompanied with a faster damage to the renal function with age. According to Bismuth et al. (2000), Pb can also induce a proximal tubulopathy observed when the contamination is important and when the damage of the kidney is chronic (after 10 to 30 years of exposure).

Renal function assessment

None of the participant was found to have chronic renal failure. Only 8.57% had a glomerular filtration rate between 60 and 89 ml/min/1.73 m^2 which indicate a mild renal failure. According to Vigan et al. (2013), the frequency of chronic renal failure (CRF) is 2.78% in hospital setting. This study has been the first to assess the content of heavy metals in herbal teas and the impact of their regular consumption on renal function in Beninese. The founding of this study suggests further study of epidemiological prevalence of chronic renal failure within the general population of Cotonou in order to conclude on the impact of heavy metals in regular herbal tea drinkers.

Conclusion

This study has measured heavy metals in 13 types of herbal teas sold in Cotonou as well as some biological markers of kidney failure in 35 regular consumers of herbal teas in Cotonou. The herbal tea samples collected in Cotonou, Benin, contained high concentrations of heavy metals such as Cadmium and Lead. It is urgent to get the attention of regular consumers on potential risks associated with high concentrations of heavy metals in herbal teas sold in Cotonou. No severe sign of kidney damage has been identified among participants. This study results recall the question about the safety of herbal teas widely used in Cotonou. This study could provide basis for further research to evaluate the global renal impact of such consumption on a long period between regular and non-regular consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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