Analysis of Heavy Metals Concentration in Kano Herbal Preparations for Major Disease Conditions

1Samali, A., 2Mohammed, M. I. and 2Ibrahim, M. B.
1Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.
2Department of Pure and Industrial Chemistry, Bayero University P.M.B 3011, Kano, Nigeria.
Email: ayubasamali@yahoo.com, mbibrahim.chm@buk.edu.ng

ABSTRACT
The use of herbal medicines for treatment, management and prevention of different disease conditions has been as old as human history. These herbs usually contained some chemical substances which are considered as beneficial, while others were not. Study of the levels of some essential (Copper, Cadmium, Cobalt, Iron, Manganese, Nickel and zinc) and toxic (Cd and Pb) heavy metals content of herbal preparations sold in Kuru market of Kano state, Nigeria was carried out. The aim of this study is to evaluate the levels of essential and toxic heavy metals in Kano herbal preparations used for commonly identified disease conditions. The samples were digested by wet digestion method with nitric acid and perchloric acids and analyzed using Flame Atomic Absorption Spectrometer (FAAS). The data obtained were statistically treated and the results presented as mean of three replicated. The range of mean concentrations of the essentials Cu, Co, Fe, Mn, Ni, Zn and toxic Cd, Pb heavy metals obtained were 4.94-10.03μg/g, 3.33-3.80μg/g, 163.04-350.95 μg/g, 57.57-65.05μg/g, 9.94-15.63μg/g, 6.86-66.59 μg/g and 1.53-3.23μg/g, 3.75-35.24 μg/g respectively. The level of contamination of the herbal preparation with the toxic heavy metals indicated 100% of the samples analyzed contained cadmium above WHO permissible limits (0.3μg/g) specified for herbal medicine and 33% of the samples contained lead (Pb) above WHO permissible limits (10μg/g) specified for herbal medicine. The gross level of contamination of these herbal preparations with Pb and Cd metals could be associated to poor adherence to quality control requirements such as current/good manufacturing practice (CGMP) and effective surveillance/enforcement. There is need for commitment for immediate action by both the herbal medicine practitioners and the relevant stakeholders in order to avert impending health hazards due to the tendency of gradual accumulation of these toxic metals in the body system of the consumers of these herbal preparations in order to attain to safe and effective herbal medicine usage in Nigeria.

Keywords: Disease conditions, Heavy metals, Herbal preparations, Kano

INTRODUCTION
The use of plants-based in the traditional medicine systems play an important role in the healthcare of two-thirds of the world’s inhabitants (IUPAC, 2008) according to documented literatures (API, 1989; But et al., 1997; Chang and But, 2001) and many more information and knowledge are scattered around in communities, families, tribes, and with local herbal medicine practitioners. The diversity of the use of herbal medicine kept no region, culture or tribe completely ignorant of the use of herbal preparation for treating one disease ailment or the other. The World Bank estimates that trade in medicinal plants, botanical drug products, and raw materials are growing at an annual rate of between 5 and 15 % (Benedum, 1998; Patwardhan et al., 2005). These have popularized herbal products throughout the world; but one of the impediments in its acceptance by all is lack of established standard quality control measures. Some of these herbal medicines may contain elements of vital importance for human metabolism, disease prevention and healing (Obianjunwa et al., 2004; Rajurkar and Damame, 1997), while on the other hand, they may as well contain non-beneficial elements which could pose health hazard to the body system due to unascertained safety and efficacy. Essential mineral element can also play a significant role in biological activity either with organic molecules such as metallo-enzymes or independently like redox or catalytic reaction (Adongo et al., 2012). The importance of the knowledge of elemental contents of herbal preparations used for different medicinal purposes deserved more attention for screening than ever before (Arceusz et al., 2010) due to high dependence on herbal preparation for medicinal purposes. The bio-toxic and poisoning effects of heavy metals occur when consumed above the bio-recommended limits and interfere with the normal body biochemistry in the normal metabolic
processes most especially when ingested in the acid medium of the stomach, they are converted to their stable oxidation states and combine with the body’s bio-molecules such as proteins and enzymes to form strong and stable chemical bonds (Duruibe et al., 2007) which results to bio-accumulation through biological chains over the years due to their persistence and non-biodegradability. Human health therefore is at risk and can be grossly affected by heavy metal intoxication if concurrent ingestion of contaminated/polluted crude herbal products are not continuously monitored and suggest for remediation strategies (Das and Singh, 2011). The tendency of developing disease ailment instead of treating existing ones are eminent if continual consumption of contaminated herbal preparations with heavy metals are used (Ernst, and Coon, 2001; Robert et al., 2008; Martena et al., 2010). Several studies have been conducted on heavy metals and macronutrient status of herbal plants however, limited and scanty information are available with respect to Kano herbal preparations despite the fact Kano state is one of the biggest commercial cities in Nigeria which has a large market for traditional herbal medicine which serve as an outlet for distribution of herbal preparations to other states. These therefore, necessitate the need for the present study. Several analytical techniques have been used to determine heavy metals in various samples of different matrices (Helaluddin et al., 2016). In the present study, the heavy metal content of the herbal preparations were determined using Atomic Absorption Spectrometer (AAS) based on the principle of absorption of UV-visible radiation at specific frequency (Skoog et al., 2007).

**Statistical Data Analysis**
All the data obtained were subjected to statistical analysis using Microsoft Excel 2010 for processing and the results were reported as mean, standard deviation and range as calculated (Rao, 1996).

**Materials and Methods**

**Sample Collection and Preparation**
The study was carried out on Kano herbal preparations used for six (6) popular disease conditions which included sexual-drive (SD), anti-malaria (AM), anti-sickel-cell (ASC), anti-ulcer (AU), anti-hypertension (AH) and anti-diabetes (AD). The herbal preparations were examined at the point of sales (Kurmi markets–Kano state), purchased, properly packaged in polyethylene bags and coded as Kn1(AD), Kn2(AH), Kn3(AM), Kn4(AU), Kn5(ASC) and Kn6 (SD), taken to laboratory, shed-dried and finely powdered using mortar and pestle.

**Sample Digestion**
1.0 g of each sample was placed into beakers and added 20 cm$^3$ of concentrated nitric acid and kept for 24 hours after which they were carefully heated on a powered electrically connected sand-bath in fume hood chamber with periodic addition of 10-20 cm$^3$ of concentrated nitric acid until the production of red nitrous oxide (NO$_2$) ceased. The samples were cooled and 2-4 cm$^3$ of perchloric acid (HClO$_4$) was added and heated until the cleared sample solution was obtained. The samples were diluted with deionized water, filtered into 100cm$^3$ volumetric flasks, made up to mark and transferred into a capped labeled plastic bottle and kept in fridge prior to analysis. The ratio of the acids; HNO$_3$:HClO$_4$ used for the digestion was 5:1 (Sneddon et al. 2006; Hseu, 2004). Blank sample was also prepared by digesting the same proportion of the reagents used in the sample digestion under the same experimental condition without the sample.

**Preparation of Standard Solutions**
Stock standards solution of the metals of interest were prepared by weighing out accurately 1.000g of the pure metal and dissolved in concentrated nitric acid and made up to mark in 1 dm$^3$ volumetric flask with deionized water or the appropriate salt of the metal weighed out as calculated in relation to the formula weight, dissolved in 1 dm$^3$ volumetric flask and made up to mark with deionized water (Dhanlal, 2000). The working standards of appropriate concentrations were prepared from the stock solutions by diluting appropriate volume with deionized water and made up to mark in 100cm$^3$ volumetric flask and used for calibrating the AAS equipment after optimization according to the standard conditions specified in Table 1.0.
Table 1.0: Standard Atomic Absorption Condition for Determination of the Heavy Metals

<table>
<thead>
<tr>
<th>Element</th>
<th>wavelength</th>
<th>Detection limit (µg/ml)</th>
<th>Slit width (nm)</th>
<th>Lamp Current (mA)</th>
<th>Nebulizer uptake</th>
<th>Gas flow (Air/acetylene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>0.02</td>
<td>0.5</td>
<td>3.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.002</td>
<td>0.5</td>
<td>3.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Co</td>
<td>240.7</td>
<td>0.005</td>
<td>0.2</td>
<td>7.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Fe</td>
<td>372.0</td>
<td>0.45</td>
<td>0.2</td>
<td>7.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.03</td>
<td>0.2</td>
<td>5.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Ni</td>
<td>232.0</td>
<td>0.04</td>
<td>0.2</td>
<td>4.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Pb</td>
<td>217.0</td>
<td>0.06</td>
<td>1.0</td>
<td>5.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>0.008</td>
<td>0.5</td>
<td>5.0</td>
<td>5ml/min</td>
<td>13.5/2.0</td>
</tr>
</tbody>
</table>

Equipment Suitability Test

Equipment suitability and method sensitivity were determined by using quality calibration standards from which calibration curves of acceptable weighted-linear correlation coefficients (≥0.995) were generated before proceeding for sample analysis.

Sample Analysis

SpectrAA 240 Model of Fast Sequential Atomic Absorption Spectrometer was used for the sample analysis where Cu, Co, Cd, Fe, Mn, Ni, Pb and Zn were determined in the digested herbal preparations according to the operating conditions specified in Table 1.0 and calibrated using the prepared standard solutions of the metals of interest followed by the blank solution run and the sample analyte. The data obtained were processed by calculating the actual concentrations in the samples analyzed using the relation:

\[ \text{Metal} (\mu g/g) = \frac{C \times V \times d.f}{W}, \]

Where C is the concentration of the sample solution in mg/L; V is the volume of the sample solutions in mL; W is the sample’s weight in grams and d.f is the dilution factor, if used (Chionyedua et al., 2015; AAS Manual, 1996).

Results and Discussion

Replicate of samples of herbal preparations used for six (6) commonly identified diseases conditions were analyzed for essential and toxic heavy metals and the results were presented in Table 2.0 (Essential metals) and Table 3.0 (Toxic metals) as mean±SD, mean and range after statistical treatment of the data.

Table 2.0: Essential Metals in Kano Herbal Preparations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cu (µg/g)</th>
<th>Co (µg/g)</th>
<th>Fe (µg/g)</th>
<th>Mn (µg/g)</th>
<th>Ni (µg/g)</th>
<th>Zn (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn1(AD)</td>
<td>4.94±0.58</td>
<td>3.77±0.02</td>
<td>350.95±0.52</td>
<td>ND</td>
<td>9.94±0.05</td>
<td>66.59±0.80</td>
</tr>
<tr>
<td>Kn2(AH)</td>
<td>5.52±0.03</td>
<td>3.33±0.02</td>
<td>164.45±0.35</td>
<td>ND</td>
<td>12.04±0.06</td>
<td>49.38±0.55</td>
</tr>
<tr>
<td>Kn3(AM)</td>
<td>10.03±0.53</td>
<td>3.60±0.01</td>
<td>216.49±0.30</td>
<td>65.05±0.55</td>
<td>11.57±0.10</td>
<td>16.83±0.60</td>
</tr>
<tr>
<td>Kn4(AU)</td>
<td>5.76±0.42</td>
<td>3.70±0.02</td>
<td>163.04±0.30</td>
<td>57.57±1.20</td>
<td>14.48±0.01</td>
<td>21.34±0.16</td>
</tr>
<tr>
<td>Kn5(ASC)</td>
<td>6.94±0.01</td>
<td>3.80±0.02</td>
<td>315.63±0.45</td>
<td>ND</td>
<td>15.63±0.03</td>
<td>18.04±0.50</td>
</tr>
<tr>
<td>Kn6(SD)</td>
<td>8.79±0.08</td>
<td>ND</td>
<td>281.19±0.25</td>
<td>ND</td>
<td>14.27±0.01</td>
<td>6.86±0.65</td>
</tr>
<tr>
<td>Mean</td>
<td>7.00</td>
<td>3.64</td>
<td>248.60</td>
<td>61.31</td>
<td>12.91</td>
<td>30.00</td>
</tr>
<tr>
<td>Range</td>
<td>4.94-10.03</td>
<td>3.33-3.80</td>
<td>163.04-350.95</td>
<td>57.57-65.05</td>
<td>9.94-15.63</td>
<td>6.86-66.59</td>
</tr>
</tbody>
</table>

Note: ND means not detected
In this study, anti-diabetic (AD) herbal preparations analyzed indicated the concentrations of essential metals Fe, Zn, Cu, Co and Ni as 350.95±0.52 µg/g, 66.59±0.80 µg/g, 4.94±0.58 µg/g, and 3.77±0.02 and 9.94±0.05 µg/g (Table 2.0) respectively. The presence of cobalt and zinc in the anti-diabetic herbal preparation is evidence that, it has potential to handle diabetic disease condition based on the facts reported by Sakurai et al. (2010) who claimed that, mechanisms involve with these metals which included, stimulation of the activities of the gluconeogenic enzymes, antioxidant properties (cobalt and zinc), stimulation of glucose uptake, glycogen and lipid synthesis in muscle, adipose and hepatic tissues and inhibition of gluconeogenesis (cobalt) handle the challenges of diabetic disease condition. Nomura et al. (2005) also reported significant decline in blood glucose of diabetic rats when treated with cobalt chloride, without any effect on plasma insulin. Higher zinc intake has also been associated with a slightly lower risk of type II diabetes according to Sun et al. (2008). However, imbalance of some essential metals could affect pancreatic islet and cause development of diabetes due to production of reactive oxygen species according to Khan and Awan (2014) this could also further decrease the insulin gene promoter activity and mRNA expression in pancreatic islet cells due to hyperglycemic condition (Valko et al., 2005; Galhardi et al., 2004; Jiang et al., 2004; Guidotti et al., 2010).

The anti-malarial (AM) herbal preparations analyzed indicated the concentrations of Fe, Cu, Co and Ni as 216.49±0.30, 12.68±0.60 µg/g, 10.03±0.53 µg/g, 3.60±0.01 µg/g and 11.57±0.10 µg/g (Table 2.0). In malaria disease condition, the role of iron is a vital requirement for optimum production of hemoglobin in order to overcome anemic condition resulted from infected red blood cell by malaria parasite (Plasmodium falciparum) and anemia in which the condition is considered as measure of the cumulative impact of malaria on the individual patient (Ekvall, 2003), in such situation iron is necessarily required for boosting the red blood cell in order to avert the anemic condition and other hematological changes such as increase in platelet count due to the high level of Plasmodium falciparum in patient with malaria (Kotepui et al., 2015). High level of iron in the anti-malarial herbal preparations analyzed boost optimum hemoglobin supply which suppress severity of malaria condition by meeting the body requirement for all age and sex groups (Kumar et al., 2008; Bashawri et al., 2002).

The anti-sickel cell (ASC) herbal preparations analyzed indicated the concentrations of Fe, Zn, Cu, Co and Ni as 315.63±0.45 µg/g, 18.04±0.50 µg/g, 6.94±0.01 µg/g, 3.80±0.02 µg/g and 15.63±0.03 µg/g (Table 2.0). Sickle cell which is also characterized by anemia and immunological disturbances are linked to essential metal deficiency, due to increased needs of many of these essential metals in the sickle cell patients according to Idonije et al. (2011) and Tagney et al. (1989). The presence of iron, zinc, copper in the analyzed anti-sickel cell herbal preparations analyzed indicated their role alleviating the crises that may result due to the disease condition, the likely presence of antioxidant in these herbal preparations (Okpuzor and Okochi, 2009) protect and enhances the red blood cell membrane for optimum functioning in sickle cell patient against generated free radicals in the body system according to Idonije et al. (2011).

The sex-drive (SD) herbal preparations analyzed indicated the concentrations of Fe, Zn, Cu, Co and Ni as 281.19±0.25 µg/g, 6.86±0.65 µg/g, 8.79±0.08 µg/g, ND and 14.27±0.01 µg/g (Table 2.0). Sex-drive is a biological need or craving that inspires individuals to seek out and become receptive to sexual experiences and sexual pleasure (Baumeister et al., 2001). Chronic illness/disorder such as cardiovascular disease, diabetes, arthritis, high-blood pressure, enlarge prostates (in men), Parkinson’s disease and cancer can have serious effect on biological drive and psychological motivation for sexual desire, functioning and response of an individual (Levine, 2003; Regan and Atkins, 2006). According to DeLamater and Sill, (2005), the use of anticoagulants, cardiovascular medications, medications to control cholesterol, and medications for hypertension by women contributed to low

Table 3.0: Toxic Heavy Metals in Kano Herbal Preparations

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Concentrations (µg/g)</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kn1(AD)</td>
<td>35.24±5.22</td>
<td>2.53±1.26</td>
<td></td>
</tr>
<tr>
<td>Kn2(AH)</td>
<td>ND</td>
<td>1.83±0.66</td>
<td></td>
</tr>
<tr>
<td>Kn3(AM)</td>
<td>9.79±0.10</td>
<td>2.79±0.30</td>
<td></td>
</tr>
<tr>
<td>Kn4(AU)</td>
<td>3.75±0.15</td>
<td>1.53±1.38</td>
<td></td>
</tr>
<tr>
<td>Kn5(ASC)</td>
<td>ND</td>
<td>3.23±1.00</td>
<td></td>
</tr>
<tr>
<td>Kn6(SD)</td>
<td>5.78±0.44</td>
<td>2.2±0.56</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.44</td>
<td>2.35</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.75-35.24</td>
<td>1.53-3.23</td>
<td></td>
</tr>
</tbody>
</table>
levels of desire, while in men, only the use of anticoagulants and medications for hypertension was related to low levels of desire. High level of Fe obtained in the analyzed sex-drive herbal preparations are required to increase blood volume for optimal sex performance, while Zn increases semen volume and testosterone level for male sexual vitality, Zn deficiency in other hand can result to infertility, impotency, or poor reproductive system development. The recommended level of zinc for male infertility is 30-60 mg per day (Rungby, 2010). Copper plays a similar role with Zn therefore its presence in these herbal preparations contributes to effective sexual activities.

The results of toxic heavy metals content of the herbal preparations analyzed indicated thirty three percent (33%) of the samples contained lead (Pb) and 100% had cadmium (Cd) above WHO (2007) permissible limits of lead (10µg/g) and cadmium (0.3µg/g) in herbal medicine. This could pose serious health hazard to human health as these metals are not friendly to most of the major organs such as kidney, liver, renal tract, cardiovascular, reproductive and others (Maobe et al., 2012; Martin and Griswold, 2009; Johnson, 1998). The uptake and bioaccumulation of heavy metals in herbs and other plants materials are influenced by a number of factors such as climate, atmospheric deposition, concentration in the soil, the nature of the soil on which the herbs are grown and the degree of maturity of the plant at the time of harvest (Lake et al., 1984). Long-term uses of treated or untreated wastewater, plants grown along heavy traffic ways, and previous dumpsites and other anthropogenic source of heavy metals which includes the addition of organic manures waste sludge, fertilizers and pesticides which may affect the uptake of heavy metals by modifying the physiscochemical properties of the soil such as pH, organic matter and bioavailability of the heavy metals in the soil are also other factors. Farmlands near heavy traffic high-ways are exposed to atmospheric pollution in the form of metal containing aerosols. These aerosols can be deposited on soil and are absorbed by plants leaves, barks and fruits (Nwachukwu et al., 2010; Liu et al., 2007; Sharma et al., 2006; Remon et al., 2005). The observed variation in concentrations of the heavy metals among the samples analyzed may be attributed to several factors such as different absorption and accumulation capacities of the toxic and essential heavy metals by the herbal materials as suggested by Singh et al. (2011) and Prasad, (2007). The use of smelting, industrial waste, processing, commercial and other anthropogenic activities could also be the contributing factors (Bergeson, 2008, ATSDR, 2005) in a commercialized city as Kano.

CONCLUSION

The outcome of the study revealed that, the herbal preparations used for common disease conditions in Kano states contained essential metals which play vital role in handling the specified disease conditions and result also indicated gross level of contaminations of these herbal preparations with Lead (Pb) and cadmium (Cd). This indicated that a potential health risk await the consumers of these herbal preparations over a period of time due to the tendency of gradual accumulation in the body system. The study therefore suggest that the Traditional Medicine Practitioners monitor their process chain from harvest, processing, storage to the sell-point in order to ensure no unforeseen sources of heavy metal contamination of their products occur and on part of government, an enforcement of regulations on herbal preparations through monitoring and evaluation surveillance on the quality of the herbal preparations are required with urgency in order to protect the health of the citizens.

REFERENCES


air act’s future?” *Environmental Quality Management;* 18: 79–84.


