Evaluation of pharmacognostical parameters and heavy metals in some locally manufactured herbal drugs

Edebi. N Vaikosen* and Gideon. O Alade

1Department of Pharmaceutical and Medicinal Chemistry, Niger Delta University, Wilberforce Island, Nigeria
2Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Wilberforce Island, Nigeria

ABSTRACT

Five heavy metals lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe) and cobalt (Co) were determined in four (4) branded Pax-herbal products - Paxherbal Health Tea®, Paxherbal Potensine®, Paxherbal Malatreat tea® and Paxherbal Black powder® using atomic absorption spectrophotometry (AAS) after acid digestion. The samples were purchased from manufacturer's (Pax-herbal) retail outlet in Yenagoa. The concentration of lead (Pb), Cd, Zn, Fe and Co in all the samples ranged from BDL to 102.200 ± 1.838 µg/g, 0.249 ± 0.010 µg/g to 2.839 ± 0.018 µg/g, BDL to 14.638 ±µg/g, 88.557 ± 1.135 µg/g to 269.074 ± 3.433 µg/g and 0.604 ± 0.002 µg/g to 9.781± 0.032 µg/g respectively. Pb content was below detection limits (BDL) in Paxherbal Health Tea® and Paxherbal Potensine®, while the amount in Paxherbal Malatreat tea and Paxherbal Black powder was 102.200 ± 1.838 µg/g and 49.528 ± 0.583 µg/g respectively. The corresponding daily intake of heavy metals in herbal drugs were below limits stipulated by regulatory agencies such - FAO/WHO, Canadian and American National Sanitation Foundation (NSF) International, except for Paxherbal Malatreat tea where the value of 418.611 µg/day (based on recommended dosage by manufacturer) was significantly above the FAO/WHO permissible limit of 232.14 µg/day, assuming an average body weight of 65 kg for an adult human.

Keywords: Atomic Absorption Spectrophotometry (AAS), Pax-herbal products, Pharmacognostical parameter, Heavy metals, Digestate.

INTRODUCTION

In the last decade there has been a steady increase in the use of herbal medicine globally. A World Health Organization (WHO) report showed that about 70 – 80% of the world population relies on non-conventional medicine which predominately of herbal sources in their primary
health care [1]. With the continuous proliferation and expansion of herbal remedies in the Nigerian market – both locally manufactured and imported (eg Tianshi, Forever, GNLD, Edmark herbal products). A number of them are registered with the National Agency for Drug Administration and Control (NAFDAC) - the Nigerian body responsible for the Regulation of drug matters. The safety and quality of these prepared herbal drugs calls for concern for health authorities, pharmaceutical industries and the public at large (that is the end user of such remedies). A number of elements are important to plants but have harmful effect at high concentrations since their various roles in physiological processes of plant largely depend on their level in plant [2]. Medicinal plants need special attention of their elemental composition because of their therapeutic importance. Therapeutic effects are due to the presence of the active compounds or secondary metabolites and are also influenced by the associated macro and micro elements contained in them [3]. Due to increase in their potential effects on human health, accurate quantitative analysis of the elemental content of the plant is very important. The prevalence of herbal medicines use is high, the prevalence and factors with its use is largely unknown [4], even among pregnant women [5]. Although it is believed to be widespread, patients and the public have been known to self-prescribe herbal medicines for health maintenance [6,7], for the treatment or prevention of minor ailments [6 - 8] and also for chronic illnesses. In many countries, the increasing use is of special concern especially because herbal medicines are not rigorously regulated, most regulation is limited to the control of specified adulterants, and contaminants such as heavy metals and microorganisms [9]. Also, the increase use is on the high level in Nigeria [10]. The large increase of herbal use as medicines by the world population requires quality controls to ensure that toxic elements are within the maximum allowable regulation limits [11].

**EXPERIMENTAL SECTION**

**Sampling**
Four (4) samples of branded locally prepared herbal drugs manufactured by Pax-herbal were purchased directly from the retail outlet of the manufacturer in Ewu, Edo State. The branded names of the products with their National Agency for Food, Drug Administration and Control (NAFDAC) numbers , Batch numbers and expiring dates, ingredients, indications, dose and method of use are as indicated in Table 1. Pharmacognostical parameters (pH, moisture content, water soluble extractives, ethanol soluble extractives) and levels of heavy metals (Pb, Cd, Zn, Fe and Co) in products as contaminants were evaluated. All the products are registered with National Agency for Food, Drug Administration and Control (NAFDAC) – the Nigerian drug product regulatory authority.

**Chemicals and Reagents**
The concentrated nitric acid and perchloric acid used were of analytical grade. They were manufactured by Merck KGa A of Germany and BDH Limited Poole England respectively. Distilled water used was double distilled (DD).

**Instrumentation and measurements**
Heavy metals were evaluated using a Varian SpectraAA 600 flame Atomic Absorption Spectrophotometer (AAS), inter-phased to a printer. A digital pH meter was used to determine pH. All instruments were calibrated before use.
Table 1: The plants contained in the various samples, their uses and dose

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>Ingredients</th>
<th>Nafdac No./Batch No.</th>
<th>EXPIRY DATE</th>
<th>Indications</th>
<th>SINGLE DAILY DOSE</th>
<th>VEHICLE OF PREPARATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paxherbal Health Tea</td>
<td><em>Persea africana</em>, <em>Mangifera indica</em>, <em>Carica papaya</em>, <em>Cassia occidentalis</em>, <em>Vernonia amygdalina</em>, <em>Morinda lucida</em></td>
<td>A7-0451L/TBG003BB</td>
<td>25/05/13</td>
<td>Malaria fever, healthy sleep pattern, healthy teeth and bones, support immune system and as blood purifiers</td>
<td>One cup twice daily</td>
<td>A cup (300 ml) of boiling water on a tea bag (0.716 g) for 5 – 10 mins</td>
</tr>
<tr>
<td>Paxherbal Potensine Powder</td>
<td><em>Zea mays</em>, <em>Glycine max</em>, <em>Capsicum frutescens</em>, <em>Fagbox</em></td>
<td>04-7535L/PP002BA</td>
<td>19/04/13</td>
<td>Pile, general weakness</td>
<td>One leveled teaspoonful (1.5 g) twice daily</td>
<td>To be chewed and swallowed</td>
</tr>
<tr>
<td>Paxherbal Malatreat tea</td>
<td><em>Tridax procubens</em>, <em>Alstonia boonei</em>, <em>Sida acuta</em></td>
<td>A7-0452L/MT002AC</td>
<td>24/03/13</td>
<td>Treatment of symptoms of malaria and all forms of fevers.</td>
<td>One cup twice daily, one cup daily for children under 12 years.</td>
<td>A cup (300 ml) of boiling water on a tea bag (2.048 g) for 10 mins</td>
</tr>
<tr>
<td>Paxherbal Black powder</td>
<td><em>Mangifera indica</em>, <em>Cheese wood</em>, <em>Mistletoe</em>, <em>Tropical almond</em></td>
<td>04-7635L/PP002BA</td>
<td>06/03/13</td>
<td>An herbal antioxidant</td>
<td>One glass (200 ml) twice daily</td>
<td>Pour one teaspoonful into One glass of water.</td>
</tr>
</tbody>
</table>

Pharmacognostical Parameters
The following pharmacognostical parameters were carried – pH, moisture content (loss on drying), water extractives and ethanol extractives.

**pH**
1.0 g of sample was transferred into 100 mL volumetric flask containing 20 mL of distilled water. It was shaken intermittently for 25 minutes, allowed to stand for 30 minutes and made to mark with the same distilled water. The pH was measured using a pH meter.

**Determination of Alcohol–Soluble Extractive**
5.0 g of the dry herbal drug powder was macerated with 100 mL of 96% alcohol in a closed flask for twenty–four hours. The sample was shaken intermittently for six hours and allowed to stand for eighteen hours, after which, it was rapidly filtered taken precautions against loss of alcohol. 20 mL of the filtrate was then evaporated to dryness in a pre-tarred flat–bottomed shallow dish at 105°C to constant weight. The percentage of the alcohol soluble extractive was calculated with reference to the air–dried drug, fifteen replicates were made [12].

**Determination of Water–Soluble Extractive**
The procedure was exactly the same as that of alcohol–soluble extractive, using chloroform – water BP instead of 96% alcohol [12].

**Determination of Moisture Content**
5.0g of herbal drug powder was weighed into a pre-tarred flat–bottom shallow dish. The drug was then dried in an oven at 105°C for 3 hrs. This was allowed to cool in a dessicator and then weighed. The process of drying in the oven was continued for 15 minutes, followed by cooling in a dessicator and weighing using an analytical balance. This process of drying, cooling and weighing was continued until a constant weight was obtained. The percentage of water loss on drying was calculated with reference to the air–dried drug [13].
Heavy Metal Analysis

Sample preparation
Sample preparation recommended by World Health Organization (WHO) - Quality Control Methods for Medicinal Plant Materials [14] was adopted. About 1.00 – 0.500 gm of herbal drug was weighed into a pre-tarred cleaned vitreous silica crucible (DIN 12904) of 75 mL, with a vitreous silica cover. To this 1.0 mL of digestion mixture [made up of 2 parts by volume of concentrated nitric acid (67%) and 1 part by volume of concentrated perchloric acid (40%)] was added to moisten, covered and transferred into an oven without exerting pressure. The crucible was heated at 100 °C for 3 hours; then increased to 120 °C and maintained for 2 hours. The temperature of the oven was then slowly and gradually raised to 240 °C (in order to avoid losses that may result from possible violent reactions). The temperature was further maintained here for 4 hours. Dry digestate was allowed to cool and redissolved with 2.5mL of concentrated nitric acid and transferred carefully into a 100 mL volumetric flask containing 20 mL of distilled water, crucible was rinsed three times with 5 mL of distilled water and made to mark. The resultant filtrate was used for the determination of the heavy metals by using Flame Atomic Absorption Spectrophotometry [14-16]. Samples were analysed and each metal measurement was done in triplicates. A blank digestate was prepared without sample and values obtained for AAS readings were subtracted from those recorded for herbal products.

Sample Preparation for Heavy Metal determination in tea infusion
About 1 gm of tea sample was transferred carefully into 100 ml of boiled water. The tea was allowed to infuse for about 5 – 10 minutes and then filtered. Filtrate was boiled for thirty (30) minutes after adding 1 ml of concentrated HCl. Cooled and made to 100 mL volumetric flask for AAS determination. A blank was prepared without sample using 1 mL of concentrated HCl, values obtained for metals of interest were compensated for in infusion by subtraction.

Standard Calibration graph
A five point calibration graph was performed for each of the heavy metal determined (Pb, Cd, Co, Zn and Fe). The prepared concentrations of heavy metal standards used for the plot varied between 0.00 mg/mL and 10.00 mg/mL depending on the absorbance of the metal. The coefficient of correlation (r) for standard calibration graph for the heavy metals ranged between 0.99978 and 0.99998

Working conditions of Atomic Absorption Spectrophotometer
The Varian SpectraAA 600 Flame Atomic Absorption Spectrophotometer (FAAS) was set to the conditions as enumerated in Table 2 below.

<table>
<thead>
<tr>
<th>Working Parameters</th>
<th>Pb</th>
<th>Cd</th>
<th>Co</th>
<th>Zn</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>283.5</td>
<td>228.8</td>
<td>213.9</td>
<td>248.3</td>
<td></td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Cathode lamp current (mA)</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Flame type</td>
<td>air-acetylene</td>
<td>air-acetylene</td>
<td>air-acetylene</td>
<td>air-acetylene</td>
<td>air-acetylene</td>
</tr>
<tr>
<td>Air flow (Lmin⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integration time (s)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

91
RESULTS AND DISCUSSION

Pharmacognostical properties

pH

The pH values of 1.0 % (w/v) were slightly acidic for all the samples and they ranged from 6.59 to 6.80. The pH acceptable range for fruits, vegetables, grasses, flowers, trees, shrubs and annual is 4.0 – 7.5, while that of food is given as pH 2 – 9 [17].

Moisture content (Loss on drying)

The moisture content of the samples ranged from 1.10 ± 0.17 (w/w) to 3.07 ± 1.04 (w/w). Paxherbal black powder had the least moisture. The highest moisture content was obtained in paxherbal health tea, which is significantly below the maximum value of 5% (w/w) [19].

Total Ash

The residue on ashing ranged between 1.47 ± 0.74 and 5.52 ± 1.17% (w/w) (Table III), they were all below the European Pharmacopoeia maximum acceptable limit of 14% (w/w) [19].

Table 3: Pharmacognostical parameters of the herbal drugs

<table>
<thead>
<tr>
<th>Products</th>
<th>pH</th>
<th>Moisture content (%)(w/w)</th>
<th>Total Ash (%)(w/w)</th>
<th>Water Soluble Extractives (%)</th>
<th>Ethanol Soluble Extractives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paxherbal Health Tea</td>
<td>6.59 ± 0.02</td>
<td>3.07 ± 1.04</td>
<td>1.47 ± 0.74</td>
<td>24.07 ± 4.45</td>
<td>16.18 ± 4.24</td>
</tr>
<tr>
<td>Paxherbal Potensine</td>
<td>6.65 ± 0.01</td>
<td>1.75 ± 0.10</td>
<td>2.05 ± 0.49</td>
<td>16.25 ± 1.04</td>
<td>54.33 ± 4.11</td>
</tr>
<tr>
<td>Paxherbal Malatreat tea</td>
<td>6.73 ± 0.05</td>
<td>2.50 ± 0.89</td>
<td>1.98 ± 0.91</td>
<td>32.82 ± 5.67</td>
<td>16.82 ± 3.39</td>
</tr>
<tr>
<td>Paxherbal Black powder</td>
<td>6.80 ± 0.12</td>
<td>1.10 ± 0.17</td>
<td>5.52 ± 1.17</td>
<td>19.99 ± 2.34</td>
<td>16.20 ± 2.25</td>
</tr>
</tbody>
</table>

Table 4: Mean Concentrations and Standard Deviation of Heavy Metals (µg/g)

<table>
<thead>
<tr>
<th>S/N</th>
<th>PRODUCTS</th>
<th>Pb (Lead)</th>
<th>Cd (Cadmium)</th>
<th>Co (Cobalt)</th>
<th>Zn (Zinc)</th>
<th>Fe (Iron)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paxherbal Health Tea</td>
<td>BDL</td>
<td>2.8±0.02</td>
<td>0.67±0.00</td>
<td>14.6±0.10</td>
<td>241.9±5.47</td>
</tr>
<tr>
<td></td>
<td>Infs</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>17.7±5.47</td>
</tr>
<tr>
<td>2</td>
<td>Paxherbal Potensine</td>
<td>BDL</td>
<td>0.32±0.01</td>
<td>9.78±0.03</td>
<td>1.19±0.05</td>
<td>269.0±3.43</td>
</tr>
<tr>
<td>3</td>
<td>Paxherbal Malatreat tea</td>
<td>102.2±1.84</td>
<td>0.25±0.01</td>
<td>0.60±0.00</td>
<td>0.41±0.01</td>
<td>88.5±1.14</td>
</tr>
<tr>
<td></td>
<td>Infs</td>
<td>24.75±2.05</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>15.5±2.24</td>
</tr>
<tr>
<td>4</td>
<td>Paxherbal Black powder</td>
<td>49.53±0.58</td>
<td>0.57±0.05</td>
<td>0.90±0.01</td>
<td>BDL</td>
<td>160.9±3.64</td>
</tr>
</tbody>
</table>

*BDL= Below detection level, Infs: Concentration of heavy metals in herbal tea infusion

Table 5: Daily intake of Heavy Metals of Herbal Products (µg)

<table>
<thead>
<tr>
<th>S/N</th>
<th>PRODUCTS</th>
<th>Pb (Lead)</th>
<th>Cd (Cadmium)</th>
<th>Co (Cobalt)</th>
<th>Zn (Zinc)</th>
<th>Fe (Iron)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paxherbal Health Tea</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>25.42</td>
</tr>
<tr>
<td>2</td>
<td>Paxherbal Potensine</td>
<td>0.95</td>
<td>29.34</td>
<td>3.56</td>
<td>807.22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Paxherbal Malatreat tea</td>
<td>101.38</td>
<td>BDL</td>
<td>BDL</td>
<td>BDL</td>
<td>63.69</td>
</tr>
<tr>
<td>4</td>
<td>Paxherbal Black powder</td>
<td>148.58</td>
<td>1.70</td>
<td>2.69</td>
<td>BDL</td>
<td>482.97</td>
</tr>
</tbody>
</table>

*BDL= Below detection level, *Daily intake is calculated based on manufacturer’s recommended dose. The daily intake for Paxherbal Health Tea and Paxherbal Malatreat tea were calculated using levels of heavy metals found in infusions.
Water Soluble Extractives
The water soluble extractives in all the investigated samples ranged from 19% - 32% (W/W). The values obtained were not less than the minimum of 13% (w/w) [18] and not less than 15% (w/w) as stipulated by the European Pharmacopoeia [19].

Ethanol Soluble Extractives
The ethanol soluble extractives ranged between 16% and 54% (w/w) for all the samples. The lowest value (16% w/w) was above the minimum of 15% recommended by European Pharmacopoeia [19].

Heavy Metals
Lead
The concentration of lead (Pb) in the all the samples ranged from BDL to 102.20 ± 1.84 µg/g. Pb content was below detection limits (BDL) in Paxherbal Health Tea® and Paxherbal Potensine®, while the amount in Paxherbal Malatreat tea and Paxherbal Black powder was 102.20 ± 1.838 µg/g and 49.528 ± 0.58 µg/g respectively. These values were comparable to Pb content in some herbal products marketed in Pakistan [19]. Muhammad Saeed et al., 2010 reported concentration of 70.1 ± 0.00 µg/g and 45.2 ± 0.02 µg/g of Pb in two branded Pakistani herbal products – Zubex® and Kushta Qali® respectively. The daily intake of Pb in these herbal remedies based on the manufacturer’s prescription was 148.58 µg for Paxherbal Black powder respectively. However Paxherbal Malatreat tea is administered as infusion. The concentration of Pb in Paxherbal Malatreat tea infusion was 24.75 ± 2.05 µg/g, while the daily intake was 101.38 µg based on manufacturer’s prescription. The daily intakes for these products were significantly low, when compared to some herbal products in the Nigerian market [20]. Orisakwe et al., (2006) reported Pb daily intake for some herbal remedies (solid dosage form) in the Nigerian market (B-Success 28 Plant – 4200 µg, Dorasine Powder – 1375 µg, Natural Power Stone – 9125 µg, Chama black Stone – 27,000 µg and U&DEE Infection Cleansing Powder – 925 µg). The daily intake of Pb for all the products investigated were significantly below the World Health Organization/Food and Agricultural Organization (WHO/FAO) provisional tolerable weekly intake of lead per kilogram body weight is 25 µg [21] – this corresponding to 232.14 µg/day, assuming an average body weight of 65 kg for an adult human. Ingested trace metals such as lead may be absorbed into the human blood stream thereby increasing the blood level of lead. The Canadian maximum permissible limit for finished herbal product is 0.02 mg/day (20 µg/day) [14]. The mean blood level intake of adults is in the range of 20 – 514 µg/day [22]. However, regular intake of Paxherbal Malatreat tea infusion may lead to an increase in blood Pb level of patient. Also, this study revealed that the concentration of Pb in the Paxherbal Malatreat tea was 102.20± 1.84 µg/g. This is considered high and direct or whole usage instead of infusion should be discouraged. Inorganic lead is undoubtedly one of the oldest occupational toxins and evidence of lead poisoning can be found dating back to Roman times [23]. At very high blood lead levels, lead is a powerful abortifacient, at low levels, it has been associated with miscarriages and low birth weights of infants [24]. Some studies have shown reduced sperm count and motility [25], others include slowing of sensory motor time in male, reduced resistance and increased mortality rate in experimental animals [26] and impairment of antibody production with reduction of immuglobin plaque – forming cells [27].
Cadmium
The level of cadmium (Cd) in this study ranged between $0.249 \pm 0.010 \, \mu g/g$ (Paxherbal Malatreat tea) and $2.839 \pm 0.018 \, \mu g/g$ (Paxherbal Health Tea)(Table 4). These values are comparable to those reported by for some herbal plants in Pakistan ($0.59 \pm 0.41 \, \mu g/g - 1.66 \, \mu g/g$) [28]. The daily intake of Cd with respect to prescription by manufacturer was BDL, 0.951 \mu g, BDL, and 1.70 \mu g for Paxherbal Health Tea, Paxherbal Potensine®, Paxherbal Malatreat tea and Paxherbal Black powder® respectively. The highest concentration of 4.065 \mu g obtained for Cd in Paxherbal Health Tea (whole tea in table 3) was below the Canadian and American National Sanitation Foundation (NSF) International proposal of 0.006 mg/day (or 6.0 \mu g/day) for finished herbal preparation and dietary supplement [14]. Also, the FAO/WHO tolerable weekly intake of cadmium per kg body weight is 7 \mu g, corresponding to70 \mu g/day for adults with average body weight of 70 kg [21]. The characteristic clinical manifestations of chronic cadmium intoxication include renal proximal tubular dysfunction, osteomalacia and anaemia. Accumulating evidence suggests that cadmium toxicity may also affect various organs such as the liver, lung, testis and haematopoietic system [29,30].

Zinc
The concentrations of zinc (Zn) ranged from BDL to 14.638 \pm 0.100 \mu g/g for all the four Pax herbal products investigated. The Zn content was below detection limit (BDL) in Paxherbal Black powder, while the highest level (14.638 \pm 0.100 \mu g/g) was found in Paxherbal Health Tea. The levels of Zn obtained in this study were significantly below those reported in herbal branded drugs marketed in Nigeria (800 – 25,000 \mu g/day) [20] and Pakistan (5.1 – 1071 \mu g/g) [19] respectively. The daily consumption of Zn based on the recommended dose by the manufacture of Paxherbal Health Tea is 20.962 \mu g. At trace level, Zn is an essential element in man and animals. It is known to play vital roles in a number of physiological activities in man. The catalytic activity of about one hundred (100) enzymes are Zn dependent in the human body and also participate in cell signaling, release of hormones and apoptosis [19,31]. The recommended dietary allowances are 4000 – 5000 \mu g/day, 9000 – 13000 \mu g/day and 13000 – 19000 \mu g/day for children, women and men respectively [32]. The National Research Council (NRC) recommended daily Zn intake is between 10,000 and 20,000 \mu g/day [22]. Although human body can accommodate high concentrations of zinc, acute Zn toxicity (oral dose of 225000 – 450000 \mu g) can cause eminent health problems such as stomach cramps, skin irritations, vomiting, nausea and anaemia, while chronic exposure could lead to copper deficiency in man [33]. Very high levels can damage the pancreas and disturb the protein metabolism and cause arteriosclerosis. Extensive exposure to zinc chloride can cause respiratory disorders. It can be a danger to unborn and newborn children when their mothers have absorbed large concentrations and the children get exposed through blood or breast milk [34].

Iron
Iron (Fe) is an essential element in man and plays a vital role in the formation of haemoglobin, oxygen and electron transfer in human body [35]. The concentrations of iron determined was $241.920 \pm 5.470 \, \mu g/g$ (346.429 \mu g/day), $269.074 \pm 3.433 \, \mu g/g$ (807.222 \mu g/day), $88.557 \pm 1.135 \, \mu g/g$ and $160.991 \pm 3.642 \, \mu g/g$ (482.973 \mu g/day) respectively for Paxherbal Health Tea, Paxherbal Potensine®, Paxherbal Malatreat tea and Paxherbal Black powder®, with daily dose based on manufacturer’s prescription in parenthesis (Tables 3 and 4). The highest concentration of iron and daily dosage intake based on prescription was found in Paxherbal
Potensine (269.074 ± 3.433 µg/g, 807.222 µg/day). The least concentration of iron was found in Paxherbal Malatreat tea (88.557 ± 1.135 µg/g), while the least daily Fe intake was recorded in Paxherbal Health Tea (346.429 µg/day). The Paxherbal Matatreat had a higher daily Fe intake than Paxherbal Health Tea because of the prescribed dose by the manufacturer (Table 3). The highest Fe consumption of 807.222 µg/day (Paxherbal Potensine) obtained in this study is significantly incomparable to values of 11,500 µg/day (U&DEE Infection cleansing powder), Dorasine Powder (18,250 µg/day) and Natural Power Stone (123,750 µg/day) earlier reported [20]. All investigated samples were below the maximum permissible level (MPL) of 1000 µg/day [22]. The Food and Nutrition Board (FNB) of the Institute of Medicine (IM) recommended dietary allowance of 7 -10 mg/day (700 – 1000 µg/day) for children, 8 mg/day (800 µg/day) for adults and 27mg/day (27,000 µg/day) during pregnancy for mothers [33]. The ingestion of large quantities of iron salts may lead to severe necrotising gastritis with vomiting, haemorrhage and diarrhoea followed by circulatory shock, also diseases of aging such as Alzeihmer’s disease, other neurodegenerative disease, arteriosclerosis, diabetes mellitus may all be contributed to by excess iron and copper [36].

Cobalt
The mean concentration of cobalt (Co) and standard deviation in samples ranged from 0.604 ± 0.002 µg/g to 9.781 ± 0.032 µg/g. The highest value was recorded in Paxherbal Potensine®, while the least was in Paxherbal Malatreat tea. A daily concentration of 29.343 µg of Co is taken in by patients based on the recommended dose by the manufacturer. Jabeen et al., 2010 reported a concentration range of 3.41 ± 0.60 µg/g and 11.26 ± 0.30µg/g for some herbal plants found in Pakistan [28]. Presently there are no regulatory limits by WHO/FAO for Co content in herbal plants and preparations. At low concentrations they play prominent role in the formation of cyanocobalmin – vitamin B 12, an essential vitamin in man. Exposure to high concentrations may lead to some adverse effects in man. Signs and symptoms of cobalt poisoning can include visual impairment, hypothyroidism, peripheral neuropathy, rashes [37], cardiomyopathy, cognitive and auditory impairment [38].

CONCLUSION
This study from a toxicological point of view, showed that these herbal preparations are safe and do not pose any likely harm to patients if prescriptions are adhere to as stipulated by the manufacturer. The daily intake of Pb, Co, Zn, Cd and Fe from these herbal preparations were below the maximum permissible elemental daily intake by FAO/WHO. The level of lead content in Paxherbal Malatreat tea for whole preparation was considered high, however the level found in infusion was significantly low 24.75µg/g (100.13 µg daily intake), which was below the maximum permissible daily intake of 232.14 µg [14].

REFERENCES
[31] AQ Truong-Tran; LH Ho; F Chai; PD Zalewski. J. Nutrit., 2000, 130, 1459S – 1466S.
[38] O Karovic; I Tonazzini; N Rebola; E Edstrom; C Lovdahl; BB Fredholm; E Dare. Biochem. Pharmacol., 2007, volume 73 (5), 694-708.