ASSESSMENT FOR THE PRESENCE OF AFLATOXINS AND HEAVY METALS IN COMMERCIALLY AVAILABLE HERBAL MEDICINES.

INVESTIGATOR:

KARIUKI ELIZABETH NJOKI

U29/2774/2008,

SCHOOL OF PHARMACY.

SUPERVISOR:

DR.FAITH OKALEBO

DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY

SCHOOL OF PHARMACY

UNIVERSITY OF NAIROBI

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ABSTRACT

It is estimated that about 70–80% of the world's population relies on non-conventional medicine, mainly of herbal origin. The idea that just because herbal medicine products come from natural sources they are completely safe is dangerously false; they are sometimes contaminated either intentionally or unintentionally in a variety of ways with toxic heavy metals, pesticide residues and microbes which impose serious health risks to consumers. It is therefore critical to screen herbal medicine for heavy metals and aflatoxins in order to ensure that they are safe for use.

The purpose of this study was to assess for the presence of heavy metals and aflatoxins and thus determine the safety of commercially available herbal medicine in the Kenyan market since it is gaining popularity especially in the treatment of chronic illnesses.

It was a laboratory based analytical study. Nine samples were obtained by convenient sampling of herbal medicine from a herbal shop and screening was carried out using Atomic Absorption Spectroscopy for heavy metals and Thin Layer Chromatography for aflatoxins. The results obtained were be fed into an Excel package for Data analysis and presented in the form of tables, histograms and pie charts.

From the results none of the samples were contaminated with aflatoxins, cadmium or copper. A significant percentage (66.7%) of the samples was contaminated with negligible amounts of lead.

These findings showed that commercially available herbal medicines lack toxic levels of heavy metals and aflatoxins. Further research is required on a larger sample size which will be a clear reflection on the prevalence of the contaminants in herbal medicine in Kenya and ascertain for sure that these remedies are safe for use.

DECLARATION

1. DECLARATION BY AUTHOR AND RESEARCHER

I hereby solemnly declare that this research project is my original work and has not been submitted for a degree in any other institution.

NAME: KARIUKI ELIZABETH NJOKI

REGISTRATION NUMBER: U29/2774/2008

SIGNATURE: .....

DATE: 12/10/2012

2. DECLARATION BY THE SUPERVISOR

I hereby declare that this research is the student's original work and has been submitted to me

For approval as the University project supervisor.

NAME: DR. FAITH OKALEBO

SIGNATURE: .....

DATE: 12/10/012

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To my parents and siblings, I can't thank them enough for their love, support and encouragement. They have been a source of profound inspiration throughout my life.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AOAC	Association of Official Analytical Chemists
HPLC	High Pressure Liquid Chromatography
ICP	Inductively Coupled Plasma spectrometry
LC	Liquid chromatography
MP	Mobile phase
NAA	Neutron Analysis Activation
Ng/g	Nanograms/grams
Ppm	parts per million
SP	Stationery Phase
TLC	Thin Layer Chromatography
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Herbal medicine use has become increasingly popular not only in the developing countries but also in developed countries. This is especially true for the treatment of chronic ailments such as diabetes, osteoarthritis, cancer, hypertension, asthma and HIV/AIDS. Currently, there are many different herbal preparations that are manufactured to treat not only chronic illnesses but also a wide range of diseases including the common cold, infertility, rashes, and pain.

This recent resurgence of interest in plant remedies has been spurred by several factors: their cost effectiveness and availability, patients' belief that their physicians have not properly identified their problem hence feel that herbal remedies are another option. Preference for natural therapies is also due to a commonly held erroneous belief that herbal products are superior to manufactured products and have minimal side effects compared to conventional medicines; this is controversial since the chemical constituents of some herbal medicines can clearly lead to toxicity (WHO, 2005).

The WHO estimates that 70- 90% of people in Kenya and other African countries use herbal medicine for primary health care (WHO, 2002). Unfortunately the number of reports of people experiencing negative effects caused by the use of herbal medicine has also been increasing; this could be attributed to poor quality of herbal medicine as a result of insufficient quality assurance. Poor handling of herbal products including insufficient drying and improper storage conditions presents a favorable environment for harmful microorganisms to grow which in turn produce toxins, a major mechanism by which microbes cause disease. Despite the WHO guidelines on maintenance of quality herbal products there is a gap between the available knowledge and implementation since the handlers of herbal medicines are not aware of these guidelines (Alwakeel, 2008). Farmers should be educated on good agricultural practices, for instance, immediate drying of the crops after harvesting and vacuum sealing of the dried material helps prevent fungal growth.

As is the case with any medicine, the patient expects a predictable effect and desirable outcome without unwanted effects but in reality, the use of any effective medicine carries certain risks along with its benefits. Unexpected and undesirable effects arise from damage caused by various toxic constituents of beneficial herbs themselves, from unintended misidentified herbs and from adulterants, dirt and other substances present as contaminants, such as herbicides, pesticides, microbes and heavy metals (Bateman et al., 1998; Ernst, 2002a).

Earlier studies have shown that besides harmful microorganisms, herbal medicines are also contaminated with pesticides, lead, magnesium and mercury. A study in Malaysia showed that 26% of the available herbal plants possessed 0.53-2.35 ppm of mercury and therefore did not comply with quality requirement for herbal medicines (Ang et al, 2006). Another study showed that 64% of samples collected in India contained significant amounts of lead, 64% mercury, 41% arsenic and 9% cadmium (Ernst, 2002b). Most herbal products are contaminated with heavy metals since unlike prescription medication they can be sold as dietary supplements and therefore are not required to undergo rigorous testing before they are released to the market.

Many consumers have embraced the use of herbal medicines as a natural approach to their health care. They are deemed to be safe and more effective than western medicine in the control of chronic illnesses. Therefore quality control of herbal products to assess its safety is of paramount importance before they are consumed by patients to prevent the lethal effects associated with these substances. There should be regulatory systems for aflatoxin monitoring and control, including stringent quality control measures for heavy metals to assure that products released to the market are safe for use. Trading premises should be inspected regularly to ensure that the herbal medicines are properly handled and stored.

Some of the parameters required for the quality evaluation of herbal medicine include: assessment for inorganic matter, absence of adulteration, microbial load, identification and profile of contents, heavy metals, pesticides and product stability. However quality control poses a challenge due to the multi-component nature of these preparations which contain not only the active ingredient but also other ingredients such as minerals, preservatives or other irrational ingredients that make quality control more complicated as it requires complicated processing to isolate and identify the chemical ingredients (Kibwage et al, 2005).

1.2 PROJECT RATIONALE

Since the use of herbal medicine has gained recognition globally, there is need to ensure that the drugs are safe for use. This is especially important for medicines that are used for chronic ailments as frequent exposure to small quantities of contaminants over a long period of time leads to bioaccumulation resulting in health hazards.

Sometimes in order to minimize production costs so as to produce cheap remedies, quality assurance measures such as good agricultural practices and good manufacturing practices are overlooked. Insufficient drying of raw materials during post harvest processing and poor storage conditions favors growth of microorganisms which produce toxins, one of the major ones being aflatoxins. Aflatoxins can be extremely dangerous even when absorbed in small quantities (Kneifler et al, 2008).

Aflatoxin contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and subtropics, where climatic conditions are conducive to fungal growth and toxin production (Aziz et al, 1998). Kenya is located in this climatic zone, thus even after importation of the herbal products which are free from contamination, the storage conditions may promote growth of the fungus.

Most herbal medicines are sold as dietary supplements thus no stringent measures to ensure that they meet the same standards as drugs and over the counter medications thus need to screen for the presence of contaminants.

Direct or intentional adulteration of drugs for instance, Ayurvedic medicine is a traditional system native to India. It stresses the use of natural plant-based medicines. Minerals including sulfur, arsenic, lead, copper and gold are often incorporated into herbal medicine for therapeutic purposes (Ernst, 2002b). Intentional adulteration may also be encouraged by traders who are reluctant to pay premium prices for herbs of superior quality. This encourages producers to sell cheaper herbs of inferior quality, but sometimes sale of inferior products may be unintentional in the absence of proper quality control.

1.3 GENERAL OBJECTIVE

The main objective was to assess for the presence of aflatoxins and heavy metals in commercially available herbal medicine in Kenya

1.4SPECIFIC OBJECTIVES

The specific objectives of the study were to screen for:

1.4.1 Aflatoxins in herbal medicines

1.4.2 Heavy metals in herbal medicines

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 AFLATOXINS

2.1.1 DEFINITION

Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus, a fungus. The most notable are *Aspergillus fungus* and *Aspergillus parasiticus*. They are best known and most intensively researched mycotoxins in the world because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans (Machida et al, 2010).

2.1.2 OCCURRENCE

The occurence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and processing periods (Michelle et al, 2011).

At least 14 different types of aflatoxin are produced in nature. Aflatoxin B_1 is considered the most toxic and is produced by both *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin G_1 and G_2 are produced exclusively by *A. parasiticus*.

Aflatoxins M_1 and M_2 are found in the milk of cows that are fed on moldy grain. These compounds are products of a conversion process in the animal's liver. Aflatoxin B1 (figure 1), aflatoxin M1, and aflatoxin G1 have been shown to cause various types of cancer in different animal species. However, only aflatoxin B1 is considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals to be identified as a carcinogen. Because aflatoxins, especially aflatoxin B1, are potent carcinogens in some animals, there is interest in the effects of long-term exposure to low levels of these important mycotoxins on human (Michelle et al, 2012).

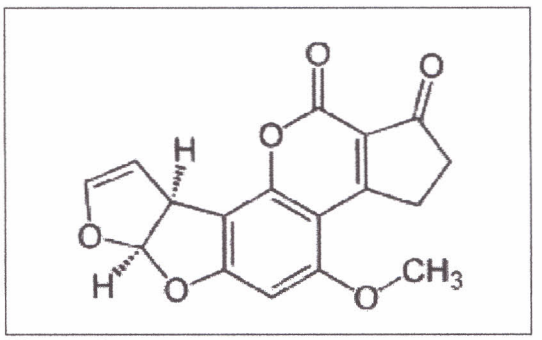

Figure 1: **Chemical structure of (–)-Alflatoxin B_1**

2.1.3 TOXICITY OF AFLATOXINS IN HUMANS

Aflatoxins have been associated with various diseases, such as aflatoxicosis, in humans throughout the world. Chronic exposure leads to a high risk of developing liver cancer. Aflatoxin's cancer-causing potential is due to its ability to produce altered forms of DNA called adducts (Montesano et al, 1997).

Epidemiological, clinical, and experimental studies reveal that exposure to large doses (>6000mg) of aflatoxin may cause acute toxicity with lethal effect whereas exposure to small doses for prolonged periods is carcinogenic (Groopmann et al 1988). High-level aflatoxin exposure produces an acute hepatic necrosis, resulting later in cirrhosis, and carcinoma of the liver. Acute hepatic failure is made manifest by hemorrhage, pulmonary edema, vomiting, abdominal pain, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart. Chronic, subclinical exposure does not lead to symptoms as dramatic as acute aflatoxicosis. Children are particularly affected by aflatoxin exposure, which leads to stunted growth and delayed development (Abbas et al, 2005)

Immuno-suppression also occurs due to the reactivity of aflatoxins with T-cells, decrease in Vitamin K activities, and a decrease in phagocytic activity in macrophages. These immuno suppressive effects of aflatoxins predispose the animals to many secondary infections (Robens et al 1992, Mclean 1995)

2.1.4 ANALYSIS OF AFLATOXINS

Several methods are used for analysis of aflatoxins: Thin Layer Chromatography is one of the most widely used separation techniques in aflatoxin analysis. It has been considered the AOAC official method and the method of choice to identify and quantitate aflatoxins at levels as low as 1 ng/g.

Liquid chromatography and TLC complement each other. Usually an analyst may use TLC for preliminary work to optimize LC separation conditions. In the analysis of aflatoxins TLC may be used for screening, thereafter HPLC for quantitative determination in samples that are positive.

Thin Layer Chromatography and Liquid Chromatography methods for determining aflatoxins are laborious and time consuming. Highly specific antibody-based tests are now commercially available that can identify and measure aflatoxins in food in less than 10 minutes. These tests are based on the affinities of the monoclonal or polyclonal antibodies for aflatoxins. The three types of immunochemical methods are radioimmunoassay, enzyme-linked immunosorbent assay, and immunoaffinity column assay (Cornell, 2011).

2.2 HEAVY METALS

2.2.1 DEFINITION

Heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Some metals such as iron are essential for life but others serve no useful biologic purpose. Examples of heavy metals that are harmful to humans include mercury, lead and arsenic.

2.2.2 TOXICITY

In small quantities certain metals are nutritionally essential for a healthy life, they become toxic when they are not metabolized by the body and accumulate in the soft tissues. Bioaccumulation of heavy metal contents through frequent years of use of these herbal medicines in chronically ill patients leads to significant serious consequences. Several degenerative and life threatening conditions are linked to accumulation of toxic metals in the body (Lynch et al, 2005).

The metals poison our bodies by competing and displacing nutritional minerals such as zinc, magnesium and calcium which are an essential part of our cellular enzymes. Disruption of activity of these enzymes interfere with cell function; disturbance of cellular redox status; and alteration of the structure of cell membranes and receptors (Katzung et al,2007)

Extensive studies have shown though that lead may have subtle subclinical adverse effects on neurocognitive function and on blood pressure at blood lead concentrations once considered normal. In key target organs such as the developing central nervous system, no safe threshold of lead exposure has been established. Lead can induce microcytic or normocytic anemia which is hypo-chromic as a result of shortened erythrocyte life span and disruption of heme synthesis (Katzung et al, 2007)

Exposure to mercury and lead may cause development of autoimmunity and this can cause joint diseases such as rheumatoid arthritis. Lead especially can cross the blood brain barrier and thus associated with Alzheimer's disease and senile dementia. Mercury can also lead to visual disturbances, paresthesias, hearing loss, mental deterioration, muscle disorders and mental disorders (Bateman et al, 1998).

Cadmium widespread exposure to the public can occur through ingestion of herbal medicine that is contaminated as a result of uptake by plants of cadmium from fertilizers and manure. Chronic exposure can lead to a serious progressive pulmonary fibrosis and severe kidney damage. Cadmium is a human carcinogen and is listed as Group 1, known human carcinogen by the IARC (Finkell et al, 2009)

Arsenic is associated with skin changes, including hyperpigmentation and hyperkeratosis involving palms and soles, peripheral vascular disease and noncirrhotic portal hypertension.

2.2.3 ANALYSIS OF HEAVY METALS

A simple straight forward determination of heavy metals based on color reactions with special reagents such as thioacetamide, and the amount present is estimated by comparison with a standard. Instrumental analysis is employed when the metals are in trace quantities or when analysis has to be quantitative. The main methods commonly used are Atomic Absorption Spectroscopy, Inductively Coupled Plasma spectroscopy, and Neutron Activation Analysis.

2.2.3.1 ATOMIC ABSORPTION SPECTROSCOPY

In atomic absorption spectrometry (AAS), light of a specific wavelength illuminates atoms in the ground state. The atoms may absorb the energy and as a result be elevated to an excited state. The amount of light energy absorbed is proportional to the concentration of atoms present in the sample. A standard solution of known concentration of atoms can be used to establish the relationship, usually by performing a standard regression analysis. It can be used for both qualitative and quantitative determination (Skoog et al, 2004).

2.2.3.2 NEUTRON ACTIVATION ANALYSIS

Neutron activation analysis is a process used to determine concentrations of certain elements in large amounts of materials by focusing on the nucleus of the elements. In NAA, radioactivity is induced by irradiating a sample with neutrons. The radioactive element formed by neutron activation decays to a stable isotope by emitting gamma rays. The rate of gamma-ray emission is proportional to the analyte initial concentration in the sample. The sensitivity of NAA varies for different elements from 0.1 - 10^6 ng/g, with heavier elements having larger nuclei being more easily activated (Harvey, 2000)

2.2.3.3 INDUCTIVELY COUPLED PLASMA SPECTROMETRY

Inductively coupled plasma (ICP) spectrometry is a technique used to determine trace amounts of certain metals. It measures the characteristic wavelength of specific light emitted by the metals when exposed to electrical currents. The electrical currents are produced by electromagnetic induction to supply energy for ICP. Argon gas is often used to create the plasma. Plasma is a conducting gaseous mixture containing a significant concentration of cations and electrons. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), can use ICP to generate excited ions or atoms that emit electromagnetic radiation at wavelengths characteristic for certain elements. The concentration of the elements can be determined by the intensity of emission. Inductively coupled plasma mass spectroscopy couples ICP to generate ions with a mass spectrometer to detect the ions. Inductively Coupled Plasma-Mass Spectroscopy offers an extremely wide detection range of elements and co-analysis of most elements in the periodic table. (Xudong et al, 2011)

CHAPTER THREE

3.0 STUDY DESIGN AND METHODOLOGY

3.1 STUDY DESIGN

A laboratory based analytical study was carried out on herbal medicines in tablet, capsule and liquid dosage forms. They were screened for the presence of aflatoxins and heavy metals.

3.1.1 SAMPLING AND SAMPLE SIZE

Nine samples of herbal medicine, five tablet, two capsule, and two liquid dosage forms were obtained from a herbal shop by convenient sampling on the basis of commercial availability and the cost.

3.2 METHODOLOGY

3.2.1 THIN LAYER CHROMATOGRAPHY FOR AFLATOXINS

3.2.1.1 MATERIALS AND EQUIPMENT

1. Sigma-Aldrich's silica gel, stationary phase -high-purity grade, Type G, with ~13% calcium sulfate, for thin layer chromatography, particle size<20microns, >632 mesh, pore size-o.7-0.85cm^3/g pore volume,60A pore size
2. Mortar and pestle
3. Chloroform- Rankem's, Analytical reagent grade
4. Acetone- Rankem's, Analytical reagent grade
5. Diethyl ether- Fischer chemical, Analytical reagent grade
6. Methanol- Sigma Aldrich's, Analytical reagent grade
7. Reference standards: Aflatoxin B_1, B_2 and G_1
8. Glass Plates
9. Distilled water
10. Wrist shaker
11. 25% sulfuric acid

3.2.1.2 SAMPLE PREPARATION

Twenty capsules or tablets of each drug were grounded and mixed with a mortar and pestle to a fine powder such that the analytical test portion had the same concentration of toxin as the original sample. Approximately 10g of the powder was extracted using 5ml of water: Chloroform mixture in the ratio of 1: 10 done in a conical flask using a solution of water and chloroform in the ratio of 1:10. The mixture was shaken vigorously using a wrist shaker for one hour. The suspensions were filtered and the filtrates evaporated to a volume of about 2ml. Samples were then diluted with two drops of chloroform: methanol (97:3) before they were spotted.

3.2.1.2 SPOTTING AND PLATE DEVELOPMENT

TLC was performed on pre-coated glass plates, (20 × 20 cm). Aldrich's silica gel was the stationary phase. Aflatoxins B_1, B_2, and G_1 were used as the reference standards. The plates were developed sequentially using diethyl ether in the first tank which cleaned the plates by separating the oils from the extract, followed by chloroform: acetone: water (88: 12: 1.5) in the second tank.

3.2.1.2 VISUALIZATION

The plates were viewed at 245nm UV light and colour of fluorescence noted. Aflatoxins standards B_1 and B_2 showed a blue colour while G_1 showed a green colour. The plates were sprayed with 25% sulfuric acid, and then observed at 365 nm under UV light and colour of fluorescence noted. The standards that were originally blue turned green and those that were originally green turned to blue. The spraying reagent was to increase the fluorescence intensity of the aflatoxins and eliminate doubtful spots since some components of the herbal drugs fluoresced under UV light.

3.2.2 ATOMIC ABSORPTION SPECTROSCOPY FOR HEAVY METALS

3.2.2.1 MATERIALS AND EQUIPMENT

Atomic Absorption spectrophotometer- GFA-Ex7i Shimadzu Graphite furnace Atomizer

1M nitric acid

crucibles

Reference standards: 1ppm, 2ppm, 3ppm, 4ppm and 5ppm of copper, lead and cadmium

Furnace

3.2.2.2 PROCEDURE

Approximately 1g of ground herbal drugs was ashed in a furnace for 3-4 hours at 525^0C to remove any organic matter. The residue obtained after burning was digested using 25 ml of 1M Nitric acid; it was added to a 100ml volumetric flask and diluted to volume with distilled water. A blank was prepared by adding 25ml of 1M Nitric acid into a 100ml volumetric flask and topped up with distilled water to 100ml.

The samples were introduced into a flame atomizer using an auto sampler. The concentration of cadmium, lead, and copper was determined by atomic absorption spectroscopy using an air–acetylene flame and external standards of known concentrations. Different lamps were used to determine the concentration of each element. The wavelengths of the lamps were 283.3nm, 228.8, 324.7nm for lead, cadmium and copper respectively.

3.3 STATISTICAL ANALYSIS

Data was analyzed using Microsoft Excel Software. The means and standard deviations were calculated and the results presented pictorially.

3.4 TIMELINE

MONTH (2012)	ACTIVITY
April	Project proposal writing
May	Carry out experiments
June	Analysis of findings, conclusions and recommendations
July	Project submission to supervisor
August	Finalizing the project and doing some corrections
October	Project presentation

3.5 BUDGET

PARTICULARS	AMOUNT (KSHS)
Herbal drugs	5000
Lab analysis costs	1000
Printing and binding costs	1500
Miscellaneous costs	500
Total	8000

CHAPTER FOUR

4.0 RESULTS

4.1 TRADE NAMES OF SAMPLE HERBAL DRUGS

Table 1 shows the name of the herbal drug, the batch number, the dosage form and the country of manufacture of the sampled drugs

Table 1: List of herbal drugs obtained from a herbal shop

Name	Batch Number	Dosage form	Country of manufacture
Septilin	D119015	tablets	India
Liv 52	D069017	tablets	India
Baariz	BRZ105	capsules	Pakistan
Neo	XNE10813	tablets	India
Plugit	138	capsules	India
Memcap	MT-9002	tablets	India
Tentex Forte	D158017	tablets	India
Pinkoo (gripe water)	0320-1	liquid	India
Memcap (syrup)	MSC-9002	liquid	India

A total of nine drugs were sampled, of these products eight were manufactured in India and one was from Pakistan. Two of the products were in liquid formulations, five were tablets and two were capsules.

4.2 EVALUATION FOR THE PRESENCE OF AFLATOXINS

The colour of fluorescence of the standards and samples observed under UV before and after spraying with 20% sulfuric acid is shown in Table 2.

Table 2: Colour of fluorescence of aflatoxins before and after spraying with 20% sulfuric acid

Sample/Standards	Before spraying (UV-254nm)	After spraying (UV-364nm)	Inference
Aflatoxin B_1	Blue	Green	+
Septilin	NF	NF	–
Liv 52	NF	NF	–
Baariz	NF	NF	–
Neo	NF	NF	–
Plugit	Blue	NF	–
Memcap	Blue	NF	–
Tentex Forte	NF	NF	–
Aflatoxin G_1	Green	Blue	+
Aflatoxin B_2	Blue	Green	+

NF =No Fluorescence, + =Aflatoxin present, – = No Aflatoxin

As shown in Table 2 none of the samples screened was contaminated with aflatoxins. Two of the samples (Plugit and Memcap) showed fluorescence before spraying, but after spraying, the fluorescence was eliminated indicating that they were false positives. The reference values for Aflatoxin B_1, B_2, and G_1 were 0.85, 0.37, and 0.52 respectively.

4.3 EVALUATION FOR PRESENCE OF HEAVY METALS

The concentrations in parts per million of Cu and Cd in the various herbal medicine samples are shown in tables 2.

Table 2: Concentration of Copper in parts per million of herbal medicine samples

Sample ID	Concentration of copper (ppm)	Inference	Concentration of cadmium (ppm)	Inference
Septilin	-0.0994	_	-0.0034	-
Liv 52	-0.0091	_	0.0000	-
Baariz	-0.0857	_	-0.0121	-
Neo	-0.0159	_	-0.0323	-
Plugit	-0.0182	_	-0.0074	-
Memcap	-0.1282	_	-0.0198	-
Tentex Forte	-0.0978	_	-0.0052	-
Pinkoo (gripe water)	-0.0207	_	-0.0224	-
Memcap (syrup)	-0.1328	_	-0.0082	-

- = no contamination

The results above indicate that the samples analyzed were neither contaminated with copper nor cadmium.

Table 3: Concentration of lead in parts per million of herbal medicine samples

Sample ID	Concentration of lead (ppm)	Inference
Septilin	0.0868	+
Liv 52	0.0256	+
Baariz	-0.0918	-
Neo	0.2195	+
Plugit	0.3777	+
Memcap	0.1634	+
Tentex Forte	0.1685	+
Pinkoo (gripe water)	-0.1888	-
Memcap (syrup)	-0.1735	-

+= Contamination with lead, - = no contamination with lead

The results above indicate that of the nine samples analyzed six of them were contaminated with lead. This represents 66.7% of all the samples analyzed.

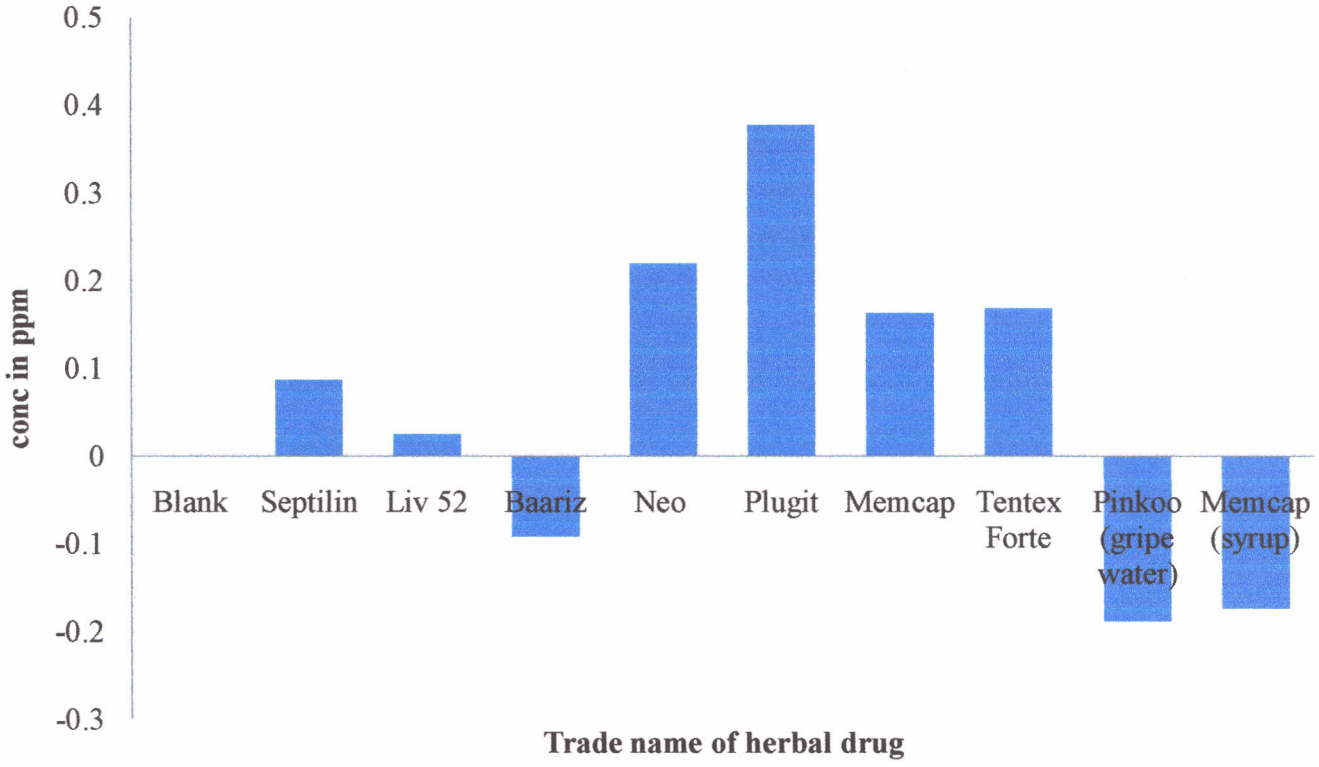

Figure 2: Concentration of lead in parts per million in herbal medicine samples

Plugit had the highest level of contamination, followed by Neo, with Memcap and Tentex Forte having almost the same level of contamination. Baariz, Pinkoo, and Memcap syrup were not contaminated with lead. In all instances of contamination, the levels were below the acceptable limits for lead (approximately 10ppm). The highest level was 0.3777ppm which is way below the acceptable limit. The mean level of contamination is 0.1736ppm and the standard deviation is +/- 0.1211

4.4 DISCUSSION

As shown in Table 2, none of the samples screened had aflatoxins. This could be attributed to adherence to good agricultural practices in the harvesting and storage of plant materials and their excipients. The drugs were properly packaged in blister packs which prevented entry of moisture. Silica gel packs were inserted in the final containers to absorb any moisture that would otherwise promote fungal growth.

Original plants could have been cultivated in soil with minimal amounts of Aspergillus since the geographical distribution of this fungus is not uniform. For instance in Kenya, aflatoxin poisoning is common in Eastern province compared to other provinces. This can be attributed not only to the poor storage conditions but also to the environmental conditions and the soil.

The sample size was too small and therefore not representative of all the herbal products sold in Kenya. The method of sampling was convenient sampling therefore the possibility of significant sampling error. A larger sample, randomly sampled from many traders in different parts of Kenya would have given a better reflection on the prevalence of aflatoxin contamination in commercially available herbal products in Kenya.

As shown in Tables 3 and 4, there was no contamination with cadmium and copper. Contamination by lead is more prevalent. Out of the nine samples screened six of them were contaminated with lead which is a large percentage. Most of the studies conducted indicate that lead was the most commonly detected heavy metal though some of the products contained significant amounts of other heavy metals (Ernst, 2002b). Therefore most herbal medicines are contaminated with lead in comparison with other heavy metals

From table 1, it is evident that most of the samples were imported from India where the practice of Ayurvedic medicine is common. Lead is added to formulations with the belief that it is an essential component of vital molecules within the human body. Numerous cases of lead toxicity associated with Ayurvedic medicine have been reported in the past. In addition to Ayurvedic medicine, other traditional medicines originating from Asian, Middle Eastern and Hispanic cultures have been found to contain lead and other heavy metals (Gogtay et al, 2002)

Contamination by lead is from a wide variety of sources including the water used in preparation of the remedies and the soil where the plants are grown, in soils with high lead content; it is possible for some lead to be taken up by the plant. Many of the herbal are sold as dietary supplements and thus do not undergo stringent quality control measures.

CHAPTER SIX

6. 1CONCLUSION

From the findings of the study, none of the samples was contaminated with aflatoxins. However, this does not imply or is not conclusive that commercially available herbal drugs in Kenya are free from aflatoxins. The sample size was too small and therefore not representative of all the herbal drugs sold in Kenya. Further studies need to be done to ascertain for sure that commercially available herbal medicines are free from aflatoxins.

It is also evident from the study that contamination with Cadmium and Copper is absent but contamination with lead is quite common. Though the levels of lead are below acceptable limits (< 10ppm, according to the British Pharmacopoeia), prolonged use of herbal medicine would result to bioaccumulation in the body, consequently the chronic lethal effects associated with this heavy metal.

6.2 RECOMMENDATION

More research is needed in order to ascertain for sure that majority of commercially available are free from aflatoxins and heavy metals since the findings of this study are not conclusive. The studies should focus on a large sample size of herbal medicines randomly obtained from all parts of Kenya.

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6.4 APPENDICES: DATA COLLECTION INSTRUMENTS

A: ATOMIC ABSORPTION SPECTROSCOPY PRINT OUT FOR CADMIUM

Action	Sample ID	True value conc.	Actual conc. (ppm)	Absorbance	Date	Time
STD		0	-0.5707	-0.0014	14-05-12	10: 14: 09
STD		1	1.0978	0.3857	14-05-12	10: 15: 01
STD		2	2.4417	0.6975	14-05-12	10: 15: 55
STD		3	3.5537	0.9555	14-05-12	10: 16: 47
STD		4	4.03	1.066	14-05-12	10: 17: 40
STD		5	4.4476	1.1629	14-05-12	10: 18: 32
UNK1	Blank		-0.5547	0.0023	14-05-12	10: 19: 25
UNK2	E1		-0.5513	0.0031	14-05-12	10: 20: 17
UNK3	E2		-0.5547	0.0023	14-05-12	10: 21: 10
UNK4	E3		-0.5668	-0.0005	14-05-12	10: 22: 03
UNK5	E4		-0.5224	0.0098	14-05-12	10: 22: 55
UNK6	E5		-0.5621	0.0006	14-05-12	10: 23: 48
UNK7	E6		-0.5349	0.0069	14-05-12	10: 24: 40
UNK8	E7		-0.5599	0.0011	14-05-12	10: 25: 33
UNK9	E8		-0.5771	-0.0029	14-05-12	10: 26: 26
UNK10	E9		-0.5629	0.0004	14-05-12	10: 27: 18

B: ATOMIC ABSORPTION SPECTROSCOPY PRINT OUT FOR LEAD

Pb

Action	Sample ID	True value conc.	Actual conc. (ppm)	Absorbance	Date	Time
STD		0	0.2034	0.0019	14-05-12	9:41:07AM
STD		1	0.7444	0.0125	14-05-12	9:41:52AM
STD		2	1.9896	0.0369	14-05-12	9:42:36AM
STD		3	3.0052	0.0568	14-05-12	9:43:22AM
STD		4	4.026	0.0768	14-05-12	9:44:07AM
STD		5	5.0314	0.0965	14-05-12	9:44:53AM
UNK1	Blank		0.0962	-0.0002	14-05-12	9:45:39AM
UNK2	E1		0.183	0.0015	14-05-12	9:46:24AM
UNK3	E2		0.1218	0.0003	14-05-12	9:47:10AM
UNK4	E3		0.0044	-0.0002	14-05-12	9:47:55AM
UNK5	E4		0.3157	0.0041	14-05-12	9:48:41AM
UNK6	E5		0.4739	0.0072	14-05-12	9:49:26AM
UNK7	E6		0.2596	0.003	14-05-12	9:50:12AM
UNK8	E7		0.2647	0.0031	14-05-12	9:50:58AM
UNK9	E8		-0.0926	-0.0039	14-05-12	9:51:43AM
UNK10	E9		-0.0518	-0.0031	14-05-12	9:52:29AM

C: ATOMIC ABSORPTION SPECTROSCOPY PRINT OUT FOR COPPER

Cu

Action	Sample ID	True value conc.	Actual conc. (ppm)	Absorbance	Date	Time
STD		0	-0.0575	0.0006	14-05-12	10: 56: 10
STD		1	0.8962	0.1263	14-05-12	10: 56: 55
STD		2	2.1414	0.2904	14-05-12	10: 57: 41
STD		3	3.1391	0.4219	14-05-12	10: 58: 27
STD		4	4.0003	0.5354	14-05-12	10: 59: 12
STD		5	4.8805	0.6514	14-05-12	10: 59: 58
UNK1	Blank		0.0813	0.0189	14-05-12	11: 00: 43
UNK2	E1		-0.0181	0.0058	14-05-12	11: 01: 29
UNK3	E2		0.0722	0.0177	14-05-12	11: 02: 14
UNK4	E3		0.0674	-0.0007	14-05-12	11: 03: 00
UNK5	E4		0.0654	0.0168	14-05-12	11: 03: 45
UNK6	E5		0.0677	0.0171	14-05-12	11: 04: 31
UNK7	E6		-0.0469	0.002	14-05-12	11: 05: 17
UNK8	E7		-0.0165	0.006	14-05-12	11: 06: 02
UNK9	E8		-0.0606	0.0002	14-05-12	11: 06: 48
UNK10	E9		-0.0515	0.0014	14-05-12	11: 07: 33

D: PHOTOGRAPH OF TLC PLATE