EVALUATION OF THE SAFETY PROFILE OF A TRADITIONAL HERBAL SUPPLEMENT *BZ 013*, USED AS AN ANTIVIRAL AND IMMUNOMODULATOR AGENT

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Declaration

I hereby declare that this is my original work and that it has not been submitted to any other institution for research or for any other reason.

Signature .....

Date 15/10/2012

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Supervised by:

Signature.....Date 15-10-2012

Professor A.N. Guantai.

Dedication

To my beloved family and friends for their love, support and encouragement.

Acknowledgement

My sincere appreciation goes to the following individuals for their support in making this project a success;

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List of Abbreviations and Acronyms

ADR - Adverse drug reaction

AST – Aspartate Aminotransferases

ALT – Alanine Aminotransferases

BUN - Blood Urea Nitrogen

DNA - Deoxyribonucleic Acid

GMP – Good Manufacturing Practices

NOAEL – No Observed Adverse Effect Level

PPB – Pharmacy and Poisons Board

SGOT - Serum glutamic oxaloacetic transaminase

WHO – World Health Organization

Abstract

Background; Toxicological tests, also known as safety screening tests or toxicity tests, are conducted to determine the degree of damage that a substance confers to living organisms. They can be used to examine the safety profile of finished materials that includes medication, chemical ingredients and food additives. They are conducted in the absence of anesthetics as there could be potential drug interactions which could affect metabolism and detoxification of chemicals by the animals which may affect the observed results. This study has indicated some potential toxicities of *BZ 013*, a traditional herbal supplement and anti viral agent, thus gives valuable information on the need for detailed toxicity studies for herbal products.

Purpose of the study; The main objective of this study was to assess the safety profile of BZ 013, a traditional herbal supplement and anti viral agent present in the Kenyan market today.

Methodology; The study was laboratory based and included the extraction of the alcohol soluble component of the drug and making a solution that was then administered to the rats at high concentration. The rats were then sacrificed after three days of administration of the test substance and observed for toxicity.

Findings and conclusions; The BZ013 was found to cause significant liver and renal toxicity. In some instances, other organs like the heart, spleen and lungs were also significantly affected. This means that the drug has potential for a lot of general body toxicity if used in high doses .

Recommendations; Larger structured studies should be carried out to generate more reliable safety information on this product and other herbal products in general. This information should then be availed to the general public and the necessary authorities before these products are released for use.

Consumers should also make an effort to equip themselves with necessary information regarding the safety of these herbal products before using them, especially those meant for long term use

1.0 CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Herbal medicine involves the use of herbs and their various parts in the treatment of infections and diseases. Herbal products are very important as they have been known to cure various types of diseases and infections ranging from bacterial, viral, parasitic and fungal infections. They have also been known to cure parasitic infestations from different microorganisms. Some examples of documented plants and their extracts known to cure include emetine from *Cephaelis ipecacuanha* for the treatment of intestinal and hepatic amoebiasis, taxol from *Taxus brevifolia* for treatment of malaria caused by *Plasmodium falciparum* and neem (*Azadiracta indica,* mwarobaini) that is referred to as the 'village doctor' due to its activity against a wide range of ailments. The contribution of these products to healthcare can therefore not be overlooked.

The use of herbal products is however faced with many challenges. As much as their use is on the increase, negative attitudes and perceptions directed at them limits their use. A sufficiently large proportion of the population still relates herbal practitioners with witchcraft and a reasonably large number modern day conventional medical practitioners still do not fully approve of the usage of herbal products. Other challenges include misconception amongst herbalists that documentation requested for by the Pharmacy and Poisons Board is intended to steal their indigenous knowledge thus hesitation to submit applications; lack of documented evidence on quality, safety & efficacy of herbal and complementary products; unethical practices that include adulteration of herbal and complementary products with conventional medicines, advertising of Herbal and complementary products in print media, electronic and bill boards; peddling of products with no therapeutic benefits; unsubstantiated medicinal claims by herbal practitioners; dealing with herbal products whose toxicological profile is not known; poor standards of preparation and sale of herbal and complementary products; and lack of adequate support for research by the government.[1]

However, there is increased awareness on the existence of these products. Traditional and modern herbal practitioners are also actively fighting for recognition from the society and the government. The government is also stepping in by helping in funding research and by registration of herbal products and the herbal practitioners. However, more can be done by incorporating measures to include quality control of these products, assisting in dose

standardization, enhancing wider utilization by encouraging use by conventional medical practitioners and by incorporating safety screening as part of the quality assurance.

The latter is crucial as of all the stated shortcomings, safety screening is key. This is done by the use of various toxicological/safety screening tests.

The toxicity tests usually examine specific types of adverse effects, known as 'endpoints'. Other tests are more general in nature and they range from single exposure (acute) studies to multiple exposure (repeat dose) studies in which animals are administered daily doses of the test substance to calculate the NOAEL. Tests aimed at identifying hazards to humans are generally referred to as "safety" or "health effects" studies, whereas wildlife and environmental tests are known as "ecotoxicity" studies.[2]

Toxicants can be classified into various groups based on their mode of action, chemical nature or class (exposure class and use class). The use class classifies drugs as therapeutic drugs, drugs of abuse, pesticides, agricultural chemicals, cosmetics, food additives and plant toxins (phytotoxins). The exposure class, on the other hand, classifies toxicants as occurring in water, food, air and soil.[3]

The tests substance can be administered through various ways that include; dermally whereby the substance is applied onto the skin; intraocular where the drug/substance is dripped into the eyes; injection through various routes that include intravenously (IV), intramuscularly (IM) or subcutaneously (SC); using the inhalation route by placing a mask over the animals and restraining them or by restraining them in an inhalation chamber; or orally by administering the test substance in their food or through a tube into their stomach. In this study, the oral route of administration will be used as the product *BZ 013* is meant for oral administration (formulated as capsules). Also this is the most convenient method for this study in terms of costs and applicability

Categories of toxicity tests

The following categories of toxicity tests have been standardized to ensure high reliability and reproducibility of the results obtained.

Acute toxicity; are carried out to determine the effect of a single dose of the test substance on a particular animal species. All the deaths caused by the test product are recorded and the changes in the dead animal, if any, including the histological, biochemical and morphological changes are investigated.

These tests are more desirable for substances that are meant for human consumption for a short duration of time, for example in the management of amoebiasis.

Subchronic toxicity; are carried out to determine the toxicity likely to arise from repeated exposure of the test substance to the test animal for a short duration of time, for example a few weeks to few months.

Chronic toxicity; these are carried out to determine the level of toxicity to an organism that occurs for a long period of time. They bear close similarity to the subchronic toxicity tests but differ in that these extend over a longer period of time and involve more animals. They are designed especially for the substances (drugs) that are meant for chronic use, for example in the management of diabetes or cancer.

Carcinogenicity; these are designed to monitor for the potential of a test substance to cause cancer. They are preferred for substances that are meant for chronic use.

Reproductive toxicity; are designed to determine the effect of a substance on an organism's gonadal function, conception, birth and the growth and development of the offspring.

Dermal toxicity; are designed to determine potential of an agent to cause irritation, inflammation or any other skin damage as a result of either direct damage by the agent or indirect response due to sensitization from prior exposure.

Neurotoxicity; these include tests for determining the effect of a substance on the organism's motor activity, peripheral nerve conduction and neuropathology.

Genetic toxicity; these are designed to measure the presence and/or extent of gene mutations, chromosome changes and DNA activity.[4]

The use of laboratory animals (in vivo) methods are however being outdated (traditional) and are being replaced by newer more convenient methods, for example in silico systems and cell/ tissue cultures.

The reasons the traditional methods are being phased out includes; they are outdated with reference to scientific progress, there is questionable reliability and relevance of the results, there is heavy investment in terms of time and costs, animal welfare guidelines and considerations limit their use as well as current legal obligations whereby some countries shave prohibited their use in cases where alternative methods can be used.[5,6]

Hepatotoxicity

Liver Injury may result from direct damage to the hepatocytes or from damage to bile canalicular cells, sinusoidal epithelial, stellate or Kupffer cells which alters the liver function or indirectly damages the hepatocytes [3]. The liver is very resilient and has regenerative properties as an adaptive response to many agents; hence, hepatic injury may not always lead to clinically decreased function. However, hepatic injury that causes functional change is of significant concern during drug development and in therapeutic drug monitoring.

Mechanisms of drug induced liver injury include

Cholestasis; Results from the inability of the hepatocytes to secrete bile as a result of impaired bile salt secretion. It can be classified into either steroid-induced cholestasis or sensitivity cholestasis. Steroid-induced cholestasis occurs mainly in C-17 substituted testosterones and the jaundice induced by these steroids is usually mild and reversible upon discontinuation of the drug. The reaction is dose related and develops after an initial period of medication. Sensitivity cholestasis is usually associated with the phenothiazines, for example chlorpromazine. It is not dose-related and develops after an initial period of sensitization of 1-4 weeks or previous exposure. The common symptoms noticed are rashes, fever, and eosinophilia and blood dyscrasias. [7]

Cytotoxic injury; Direct damage to hepatic parenchyma may be caused by different drugs through a variety of underlying mechanisms. Paracetamol causes predictable centrilobular hepatic necrosis in experimental animals and in man after overdose. The liver damage can be predicted and is due to direct cytotoxicity from an active metabolite, N-acetyl-p-benzoquinone imine. Following normal doses, this is detoxified by conjugation, both chemical and enzyme catalyzed, with tripeptide glutathione and then excreted as the N-acetylcysteine derivative in urine. However, following an overdose, the amount of reactive metabolite is sufficient to deplete the available hepatic glutathione. In this case, it reacts covalently with cellular macromolecules. Isoniazid and iproniazid which are substituted hydrazine drugs may produce hepatocellular damage in a similar mechanism.

Mixed cytotoxic/cholestatic injury; It includes damage with varying proportions of cytotoxic and cholestatic involvement. Examples include chlorpromazine and P-amino salicylic acid.

Interference with bilirubin transport and conjugation; Drugs can interfere with bilirubin transport, leading to elevated plasma bilirubin levels, a condition known as hyperbilirubinaemia. Novobiocin inhibits UDP glucuronosyltransferase and may lead to elevated plasma levels of unconjugated bilirubin especially in neonates. Rifampicin inhibits both uptake and excretion of bilirubin in a dose related manner, causing elevated plasma levels of both conjugated and unconjugated bilirubin. This is due to blockade of uptake at the plasma membrane of the hepatocytes.

Steatosis (fatty liver); Tetracycline may cause steatosis after large intravenous doses. The toxic effect of tetracycline is direct, predictable and dose dependent. The major effect is due to inhibition of transport of lipid out of the hepatocyte. This effect may be due to the inhibition of protein synthesis caused by tetracycline that inhibits the production of the apolipoprotein complex involved in transport of the very low density lipoprotein (VLDL) out of the hepatocyte. Other mechanisms may involve decreased fatty acid oxidation, increased triglyceride uptake or increased fatty acid uptake.

Phospholipidosis; this is a syndrome that may be caused different drugs whereby various organs may be affected. Hepatic phospholipidosis may be caused by the drug Coralgil (hexestrol bis (beta-diethylaminoethyl ether), a coronary dilator. The features of this form of hepatic damage

are an accumulation of phospholipids in hepatocytes, bile duct proliferation and inflammation in the portal area. The mechanism is thought to involve the formation of complexes between lipid micelles or liposomes, and the drug in question. The interaction between phospholipids and the drug is believed to alter the surface charge of the phospholipid micelle or liposome in such a way that the ability of phospholipases to break them down is impaired. [7]

Nephrotoxicity

The kidney is a major body excretory organ thus can be easily affected by the excreted substances. It is involved in the elimination of various metabolic wastes including the elimination of drugs.

Drug toxicity in the kidneys can be cumulative, causing predictable and dose-dependent effects or idiosyncratic, causing dose-independent effects during therapy.

Patterns of drug-induced renal injury include

Tubulointerstitial disease

Includes acute tubular necrosis, acute tubulointerstitial nephritis and chronic tubulointerstitial nephritis.

Glomerular disease

Includes minimal change disease, focal segmental glomerulosclerosis and membranous glomerulonephritis.

Vascular disease

Includes vasculitis/necrotising glomerulonephritis, thrombotic microangiopathy and hyaline arteriolosclerosis.[8]

Mechanisms involved in drug induced nephrotoxicity

Direct cytotoxicity

Either direct cellular toxicity or impairment of renal blood flow can injure the tubular epithelium with the effects generally being dose dependent. Aminoglycosides and many antivirals are cellular toxins which can inhibit mitochondrial function or produce intracellular free radicals.

Alternatively, interference with renal haemodynamics can be caused by non-steroidal anti-inflammatory drugs (NSAIDs) via prostaglandin inhibition, by ACE inhibitors by decreasing perfusion pressure, or by ciclosporin through afferent arteriolar constriction.

Immunological reactions

Drugs act as haptens and create antigenicity after binding to the tubular basement membrane or the interstitial matrix. This leads to T cell activation and release of interleukins which activate cytotoxic T cells and B cells leading to chronic inflammatory and changes e.g. nephrotoxicity induced by methicillin.

Mechanical effects

Some drugs have a low solubility and tend to form crystals when they are present at high concentrations in the glomerular filtrate for example sulphonamides. The crystals mechanically block the renal tubules causing crystalluria. [8]

WHO guidelines on the minimum requirements for the registration of herbal products

The WHO has come up with guidelines for the requirements in the registration of herbal products to guarantee their quality, safety and efficacy. These requirements are; Proper categorization of the herbal medicines in order to promote a harmonized assessment; Submission of the safety data for these products; Quality control of herbal medicinal products; Pharmacovigilance of these products and the control of advertisements of these products.[9]

Kenya national guidelines on the registration of herbal products

The Kenyan government, through the PPB, has also formulated guidelines on the minimum requirements that need to be submitted for the registration of these products.

These include the particulars of the product, the details of the manufacturer including the GMP of the manufacturing site, and the composition of the active and non-active ingredients of the product (excipients).

The quality control of the raw materials and the finished products should also be carried out. The stability studies of the finished products should also be availed and also the toxicological and pharmacological information. The latter should include toxicity studies of the product, adverse/side effects and the contraindications, warning and precautions. [1]

1.1 Justification

There has been a drastic increase in the use of herbal medicine in the world today. This can be attributed to by the increase in reemerging and new diseases, especially those whose treatment has not been well established. It can also be attributed to lifestyle changes and diseases that have overwhelmed the current conventional medication. Lack of adequate healthcare facilities especially in the rural areas has also forced the societies involved to look for alternative sources of healthcare.

However, there are still many challenges facing these herbal medications, as noted earlier. The greatest of these is the lack of adequate safety data. There thus needs to be established reliable and standardized safety screening mechanisms for these products.

The aim of this study is to assess the safety profile of *BZ 013* with reference to its hepatotoxic and nephrotoxic properties.

1.2 Objectives

Broad objective

To determine the safety profile of *BZ 013,* a traditional herbal nutritional supplement.

Specific objectives

I. To observe the morphological changes conferred on the major body organs upon administration of the test substance.

II. To determine the toxicity profile by performing biochemical and histopathological investigations on the major target organs, the liver and the kidney, by assessing the levels of liver transaminases and serum creatinine levels respectively.

2.0 CHAPTER 2: MATERIALS AND METHODOLOGY

A total of ten rats were used to screen *BZ 013*, a herbal anti-viral and immunomodulatory agent, for its safety profile with respect to potential to induce hepatotoxicity and nephrotoxicity. Any other morphological changes on other major body organs like the heart, lungs and the spleen were also noted.

The weight of the powder from 50 capsules of the drug were measured using a weighing balance (*Shimadzu*) after which extraction was done using 70% ethanol. The extraction was done five times successively and the extract dried using a rotary evaporator(*Laboport*). The dried extract was then weighed (0.5051g) and topped up with distilled water to 15ml to make a solution, whose strength was ~33.7 mg/ml.

The recommended human dose for *BZ 013* is 9 capsules per day in three divided doses, which translates to about 1g per dose (3 caps) in a 70 kg man. [11] This translates to 43mg/kg body weight.

The number of rats used was ten of which four were used for the test using *BZ 013* and three were used for each of the controls. Both sexes of the rats were used.

The rats were weighed and a marker pen used to mark their tails. Gavage tubes were then used to administer 1ml (33.7 mg) of the prepared drug solution orally to each of the four test rats every day at the same time for a period of three days. As the average weight of a rat was 225 g, this means that the recommended dose for the rats with respect to weight would be 3.21 mg /rat. This means that the dose given was about 11 times the recommended dose.

A positive control was set up using three rats, whereby each rat was treated with 20% Carbon tetrachloride at a dose on 20% weight of CCl_4 per weight of rat. This was done for a period of three days.

A negative control was also set up using three other rats whereby the rats were not treated with any substance. These two control groups were used for comparative purposes. It should be noted that only one dose level was used for the test and positive control.

After three days of treatment, all the animals were sacrificed by cervical dislocation and blood samples obtained by cardiac puncture. This blood was collected in vacutainers with serum clot

activator , (*Greiner bio-one)* and taken to the laboratory whereby they were centrifuged (*Minor*). The plasma was analyzed for levels of the transaminases ALT(SGPT), and AST (SGOT) to check for hepatotoxicity. Serum creatinine and urea levels were also checked to monitor for any signs of nephrotoxicity.

Photographs of the major organs (liver, kidney, heart, lungs and spleen) were taken to check for and document any morphological changes. Weights of each of these organs was also noted.

The organs were then rinsed and fixed with 10% formaldehyde and sent to the laboratory for histopathological analysis. Results were not received for inclusion in this write up due to delays from the lab.

2.1 Ethical considerations

Animals were kept under normal laboratory conditions and with free access to food and water.

The use of the rats in the study was done according to the international guidelines on animal welfare. [10]

2.2 Study limitations

The study was limited by inadequacy of resources that affected the sample size in terms of the rats to be used. This also limited the laboratory investigations to be carried out on the animals.

Time was also an issue as *BZ 013*, being a product meant for both short term and chronic use, warranted a study for both acute and chronic toxicity testing. However, only the former was possible due to time and economic constraints.

2.3 Data analysis and management

The parameters to be captured included the weights of the rats before and after the study, the weight of the extract obtained, the morphological changes on the major organs, any incidence of death caused by the product and changes in biochemical parameters. This information was captured in a predesigned data collecting instrument and later analyzed.

The data was analyzed using Microsoft Excel and the information represented on bar graphs. The parameters analyzed were presence of mortality and ill health, weight/body mass changes, organ-body mass ratio, morphological changes on body organs and the biochemical markers of liver and kidney functions.

Photographs were also used to document the morphological changes on the various body organs.

3.0 CHAPTER 3: RESULTS AND DISCUSSION

BZ 013 is a traditional herbal supplement consisting of a combination of various medicinal plant extracts including Peruvian bark, Club mosses, Purple coneflower, Bergenia ciliate, Southernwood and White hellebore.[11]

It is an antiviral and immunomodulator, whose immunomodulatory effect strengthens the immune system of the body and increases levels of CD4 and T Lymphocytes. It enables to fight against opportunistic infections and has proven effective in improving the general well being and quality of patients with chronic viral infections. [11]

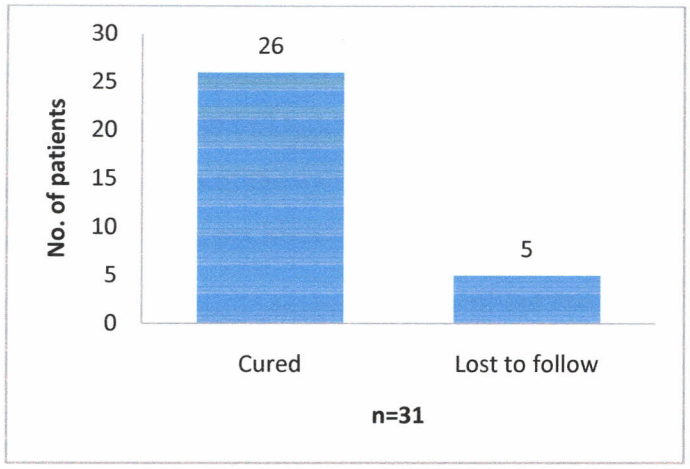
It is available in oral capsule form; adult dose established from clinical trials for *BZ 013* is 9 capsules/day in divided doses to be taken with or without food.

It is reported to have been used safely in children and when used with other antiviral therapies. No drug-drug interactions have been noted. Its use in pregnancy and lactation has not been studied. [11]

The effectiveness of this drug upon clinical trials with 31 HIV/ AIDS patients showed a cure rate of 26 patients (83.87%). This clinical trial was initiated in accordance with the ICH-GCP guidelines and in consultation with the Japanese collaborators . [12,13]

The results obtained can be graphically represented as;

Figure 1; A bar graph showing the treatment success rates for *BZ 013*



Data obtained from the internet. [11]

Table 1: Weights of drug before extraction

	Weight in grams
Weight of 50 BZ 013 capsules	19.90
Weight of BZ 013 powder equivalent to 50 capsules	16.5590

Table 2: Weights of drug after extraction

	Weight in grams
Weight of drug + beaker	32.9005
Weight of beaker	33.4096
Weight of drug	0.5051

3.1 *BZ013* Drug induced mortality

Mortality refers to death or a fatal outcome. With reference to this study, it was determined using the Lethal dose 50 (LD_{50}), which is the dose of the test substance that, in a single dose, causes the death in 50% of the animals in which it has been administered. The lethal dose is usually recorded as dose per kilogram of body weight, for example, in mg/kg of body weight.[14]

No mortalities were observed on any of the rats on *BZ 013* after the three day period of the drug administration, hence in this case, the dose was below the Lethal dose 50.

For the positive controls, the same observation of no mortality was observed. Thus the dose administered was also below the Lethal dose 50.

3.2 *BZ013* Drug induced ill health

Health, by the WHO model, can be defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO, 1946).

Ill health, the state of poor health, in this study was observed using the effect of the test substance on the mobility of the test animals (rats).

Tolerance is the capacity of the body to endure or become less responsive to a substance (as a drug) or a physiological insult especially with repeated use or exposure(Merriam Webster Dictionary, 24/09/12, 8.50pm) However, in regards to this study, tolerance shall be defined as the absence of unpleasant side effects to the test substance, such as anorexia, general malaise, pain and vomiting among others. Therefore, I was unable to determine tolerance in this study as the data on food consumption was not collected and the other parameters were difficult to ascertain.

The Maximum tolerated dose, MTD, is the highest dose of a drug or treatment that does not cause unacceptable side effects. The maximum tolerated dose is determined in clinical trials by testing increasing doses on different groups of people until the highest dose with acceptable side

effects is found. (National Cancer Institute, , 24/09/12, 8.55pm). Doses beyond the MTD lead to the unacceptable side effects of the test substance.

The MTD can be way below the Lethal dose 50 (LD_{50}), in which case the unpleasant side effects are observed in the absence of mortality. However, with very toxic substances, the MTD can be equal to the Lethal dose 50 (LD_{50}).[15,16]

In this study, no death or other unpleasant side effects were physically observed. The dose administered could be said to have been below the MTD.

3.3 Weight/body mass changes observed on the *BZ013* treated rats

Table 3: Effect of the *BZ013* test drug on body weight of the test rats

Rat No.	Weight before drug administration (In grams)	Weight after drug administration (In grams)	Percentage change in weight
1	210	203	-3.3%
2	267	248	-7.1%
3	207	173	-16.4%
4	212	189	-10.8
Average	225	203	-9.4%
Mean	225	203	
SD	24.8898	27.9318	

Rat 3 was the animal that was most affected, it showed the greatest loss in body weight at 16.4% (1 s.f.) while rat 1 was observed to be the least affected at 3.3%. Rat 3 also showed significant loss in body weight .

A % loss of weight of 10 or more is usually a sign of toxicity. With regards to this, then the test substance caused significant levels of toxicity to rats 3 and 4. However, it should also be noted

that body weight changes could be as a result of anorexia or other pathological changes thus this test parameter is not conclusive, but rather, more of a guide to indicate possibility of toxicity.

A % loss of weight of less than 10% is on the other hand an indicator of no toxicity. With regards to this, then there was no toxicity caused by the test substance in rats 1 and 2. However, this parameter is also not conclusive of the evidence of toxicity, but a guide to indicate possible absence of toxicity. More supporting evidence like the morphological changes and biochemical markers should be used to support the conclusion.

The mean % loss of weight of the whole group stood at 9.4 (1 s.f), indicating relatively that this group of rats did not suffer significant toxicity. However, as noted earlier, this only serves as a guide and should be supported using more evidence.

Figure 2; A comparative bar graph showing individual changes in weights for the rats

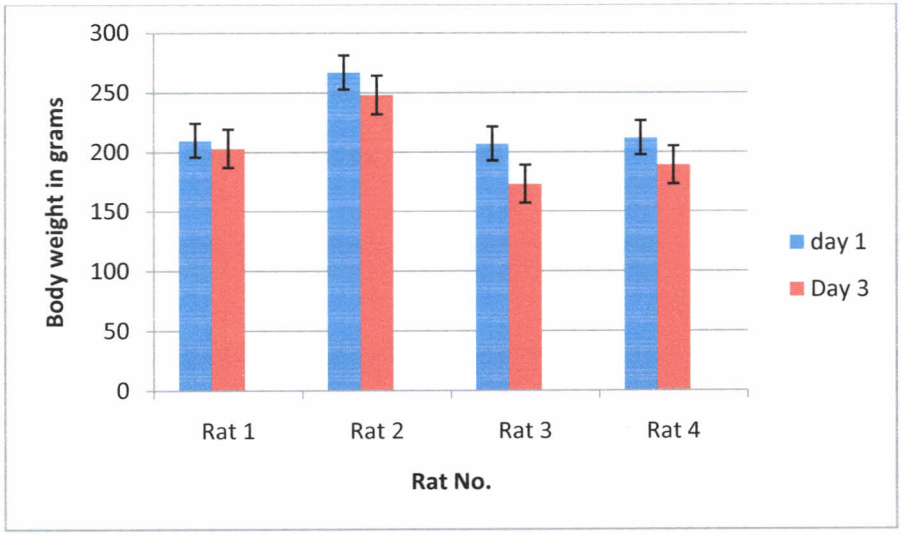


Figure 3; A graph of mean body mass against number of days

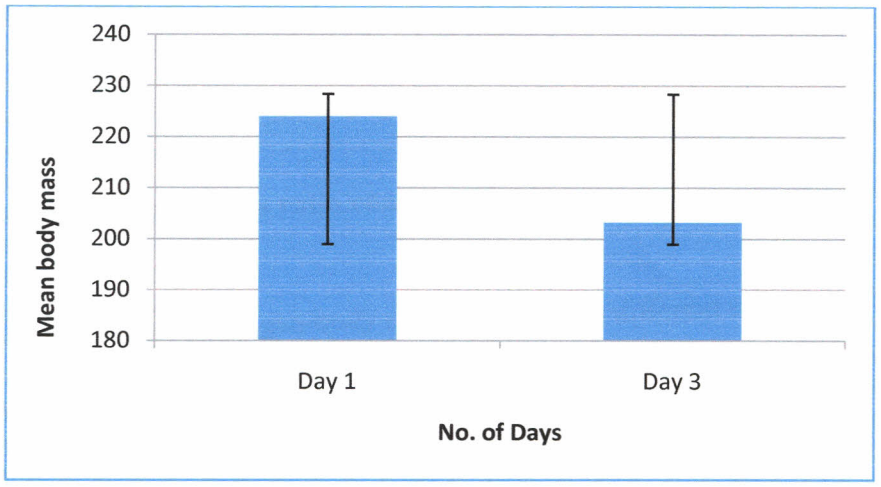


Table 4: t test reflecting on the body mass changes

t-Test: Two-Sample Assuming Equal Variances		
	Day 1	*Day 5*
Mean	224	203.25
Variance	826	1040.25
Observations	4	4
Pooled Variance	933.125	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.96064557	
P(T<=t) one-tail	0.18691388	
t Critical one-tail	1.94318027	
P(T<=t) two-tail	0.37382777	
t Critical two-tail	2.44691185	

From the paired one sided student t test, the P value is greater than 0.05, therefore the changes in body weight were not statistically significant. This means that there is no significant difference in weight of the rats between day 1 and day 5.

3.4 Organ-body mass ratio changes of rats

The changes in the organ-body mass ratio for the different rats can be interpreted as following;

An increase in this ratio is an indication of inflammation or a change in the tissue type. For example, the administration of carbon tetrachloride causes liver cirrhosis whereby the normal liver parenchyma is replaced by collagen tissue, which is reflected as an increase in organ body mass ratio.

A decrease in the ratio could be an indication of tissue necrosis, destruction or under perfusion.

Table 5: The average values for the organ-body mass ratios from the experimental results

Organ	**organ-body mass ratio**			**Mean**	**SD**
	***BZ013* Drug**	**Negative control (Normal)**	**Positive control**		
Lungs	0.0344	0.0110	0.0071	0.0175	0.014765
Heart	0.0039	0.0041	0.0038	0.0039	0.000153
Kidneys	0.0074	0.0057	0.0076	0.0069	0.001044
Spleen	0.0046	0.0041	0.0039	0.0042	0.000361
Liver	0.0397	0.0454	0.0442	0.0431	0.003005

Table 6: t-test reflecting of the organ body mass changes

t-Test: Two-Sample Assuming Equal Variances		
	Drug	*Normal*
Mean	0.018	0.01406
Variance	0.00030765	0.00031496
Observations	5	5
Pooled Variance	0.0003113	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.35308062	
P(T<=t) one-tail	0.36657545	
t Critical one-tail	1.85954803	
P(T<=t) two-tail	0.73315091	
t Critical two-tail	2.30600413	

From the paired one sided student t test, the P value is greater than 0.05, therefore the changes in organ body mass ratio were not statistically significant. This means that there is no significant difference in weight of the rat organs with or without the administration of the drug.

Table 7: Organ body mass ratios for the test rats

Organ	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**
Lungs	0.0082	0.0059	0.0115	0.0120
Heart	0.0032	0.0042	0.0042	0.0040

Kidney	0.0068	0.0075	0.0070	0.0082
Spleen	0.0044	0.0052	0.0044	0.0044
Liver	0.0476	0.0335	0.0407	0.0368

Figure 4; A comparative bar graph showing organ body mass changes for the rat 1

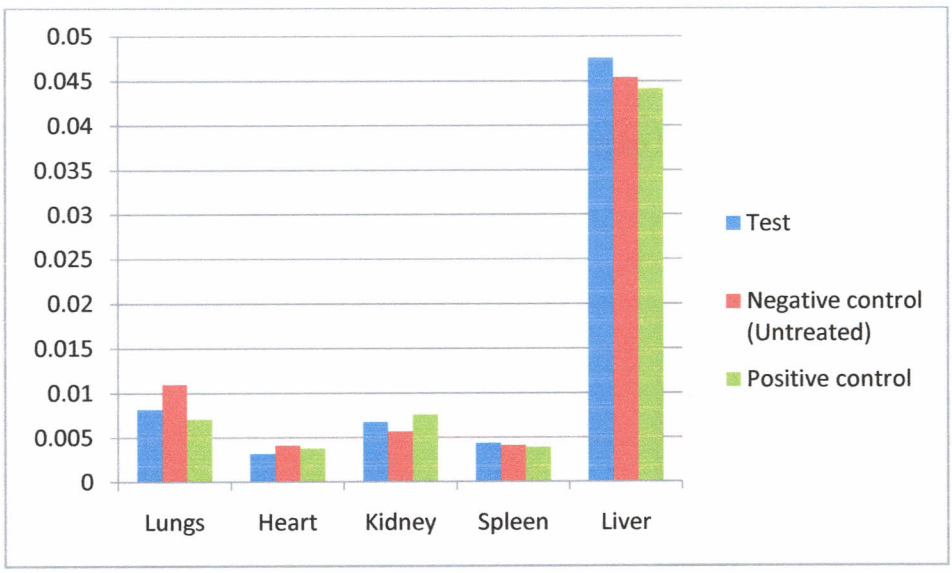


Figure 5; A comparative bar graph showing organ body mass changes for the rat 2

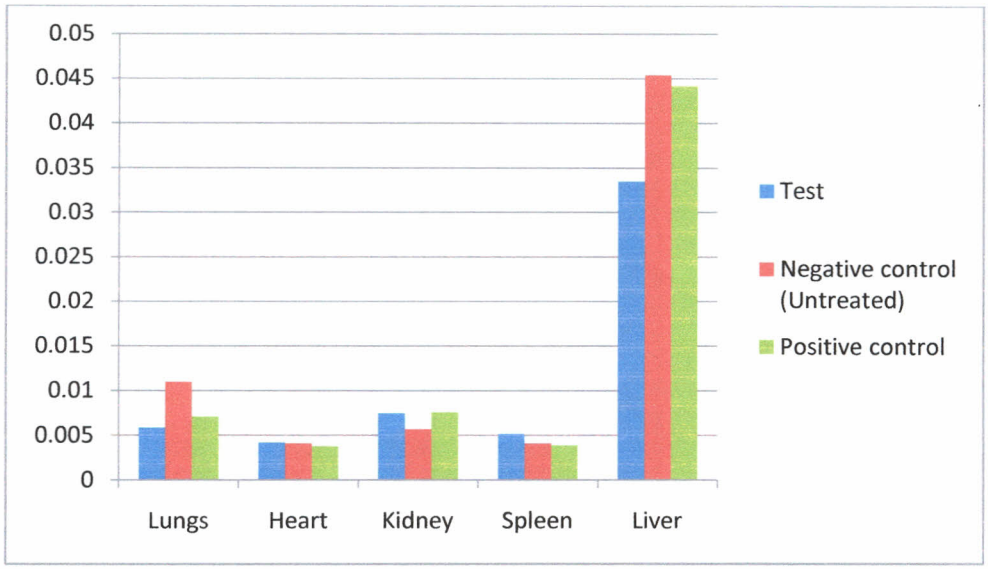


Figure 6; A comparative bar graph showing organ body mass changes for the rat 3

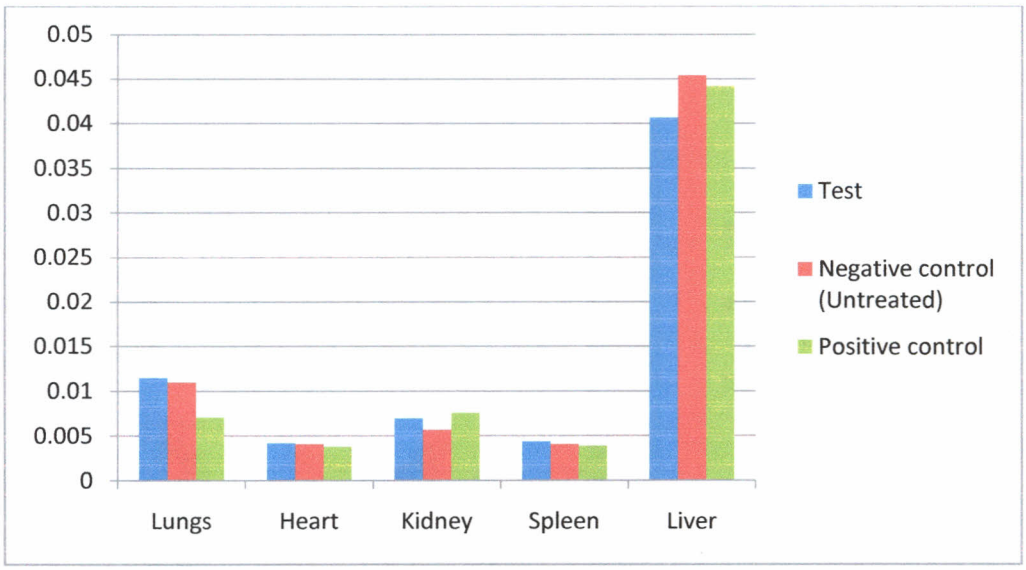


Figure 7; A comparative bar graph showing organ body mass changes for the rat 4

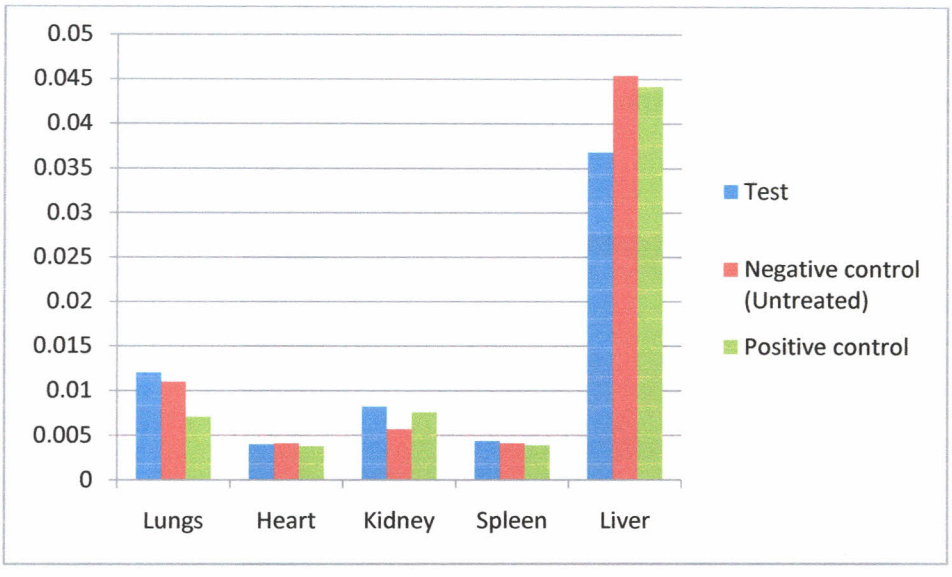
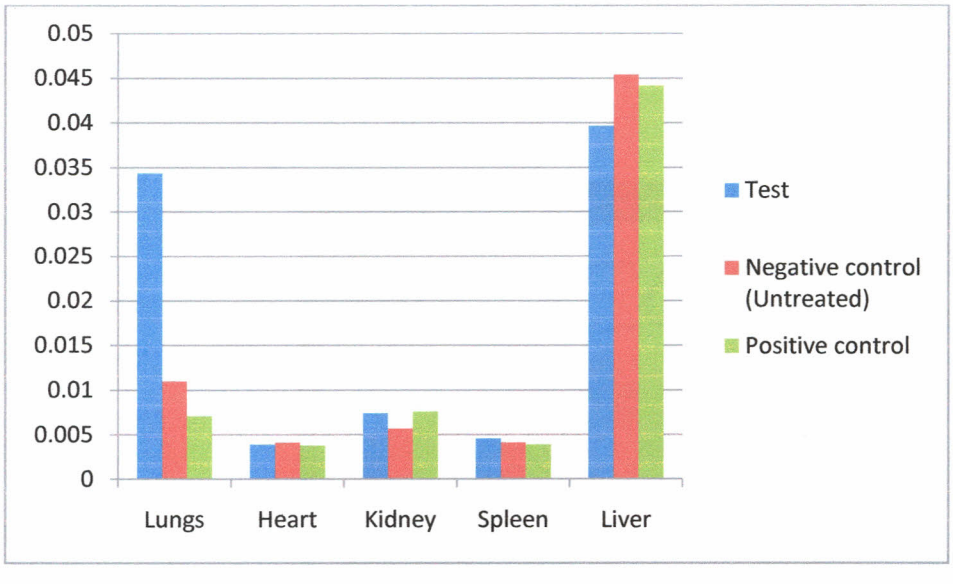


Figure 8; A comparative bar graph showing average organ body mass changes for the whole group

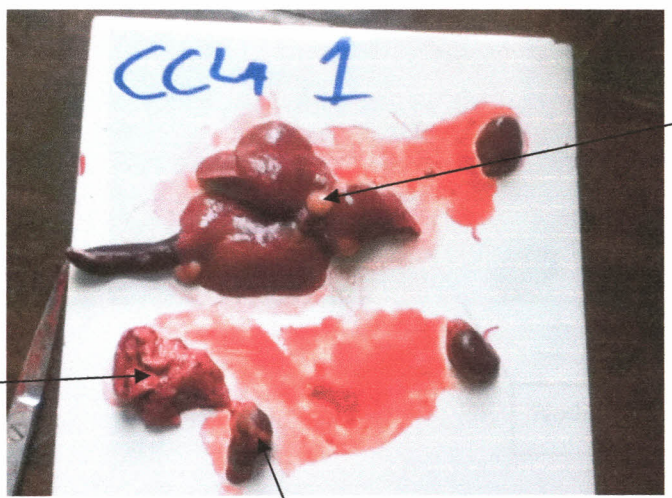


3.5 Morphological changes to body organs

Positive controls, on Carbon tetrachloride

Figure 9; Photographs showing morphological changes on rat 1,2 and 3

Rat 1

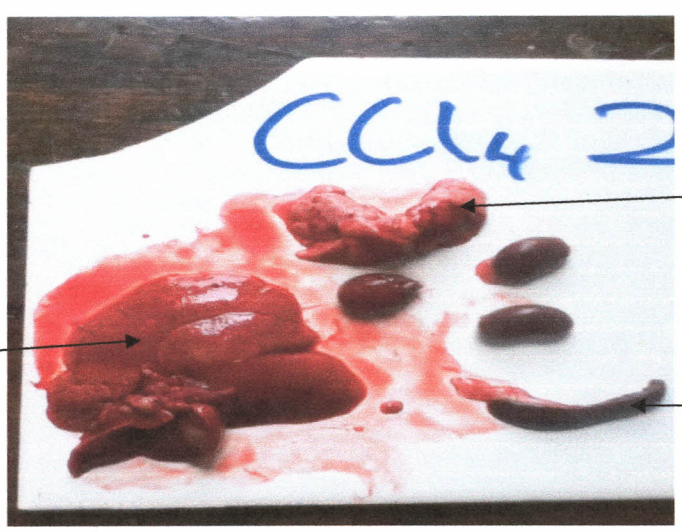


Fluid filled lesion on liver

Pale lungs

Fibrous layer on kidney

Rat 2



Pale lungs with mottled appearance

Liver with mottled appearance

Darkened spleen

Rat 3

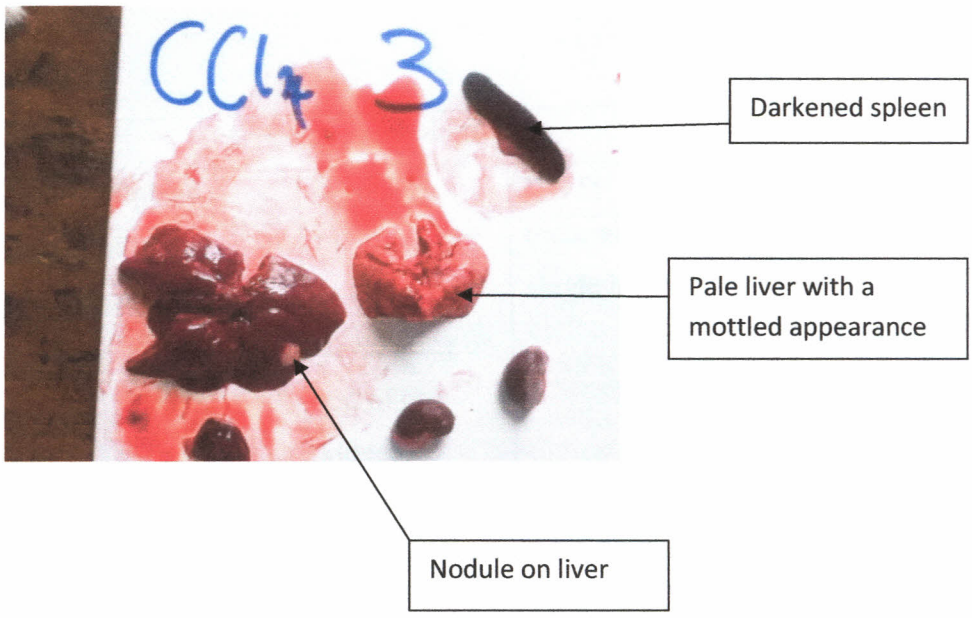

CCl4 3
Darkened spleen
Pale liver with a mottled appearance
Nodule on liver

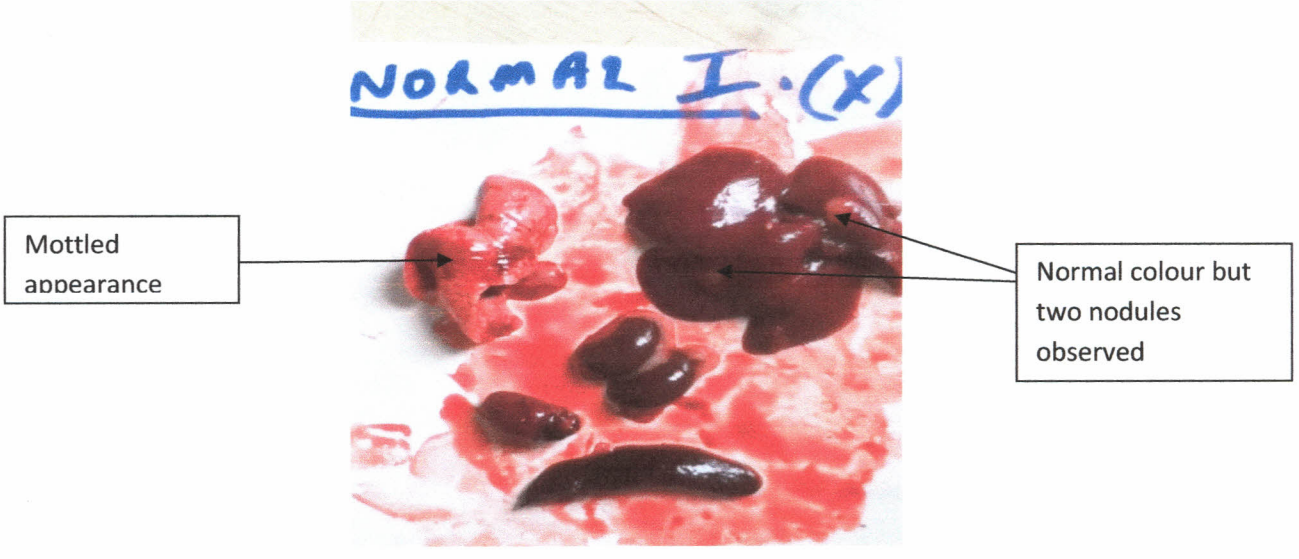
Table 8: Morphological changes observed on the various internal organs for the positive control rats

Rats	Organ morphology				
	Liver	**Spleen**	**Kidneys**	**Heart**	**Lungs**
R1	Nine yellow coloured nodules noted, one fluid filled. Liver easy to cut. Mottled appearance.	Slightly darkened.	Fibrous layer noted developing on top of one kidney.	Presence a single lesion.	Pale than normal.
R2	Two nodules noted. Mottled in appearance.	Slightly darkened.	Normal in appearance.	Normal in appearance.	Pale with red patches, mottled appearance.
R3	One nodule noted. Mottled appearance.	Darkened in colour.	Fibrous layer noted developing on one kidney.	Normal in appearance.	Pale in appearance.

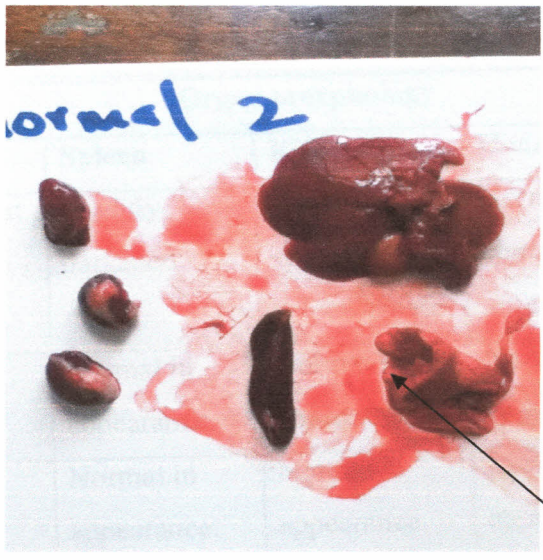
Negative controls(untreated), no active substance administered

Figure 10; Photographs showing morphological changes on rats 1,2 and 3

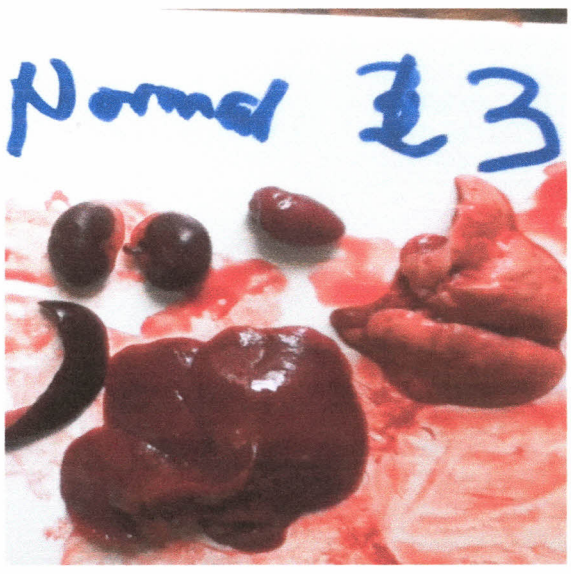
Rat 1



Rat 2



Rat 3



All organs normal.

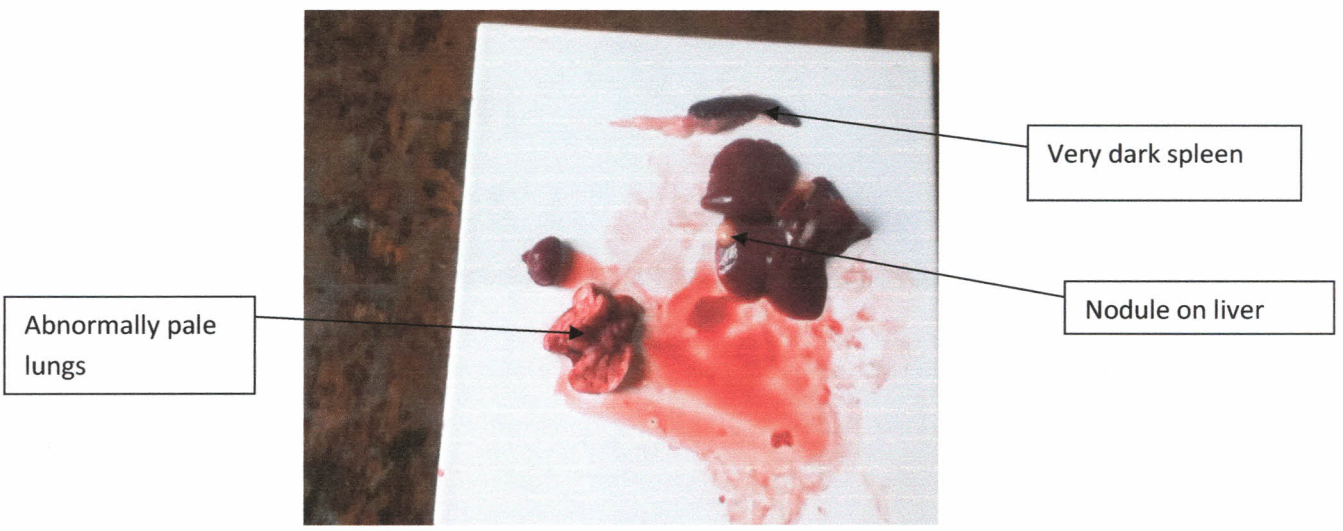
Table 9: Morphological changes observed on the various internal organs for the untreated rats

Rats	Organ morphology				
	Liver	**Spleen**	**Kidneys**	**Heart**	**Lungs**
R1	Normal in colour, but 3 nodules were observed.	Slightly darkened.	Normal in appearance.	Normal in appearance.	Mottled appearance.
R2	Normal in appearance.	Normal in appearance.	Normal in appearance.	Normal in appearance.	One nodule noted .
R3	Normal in appearance.	Normal in appearance.	Normal in appearance.	Normal in appearance.	Normal in appearance.

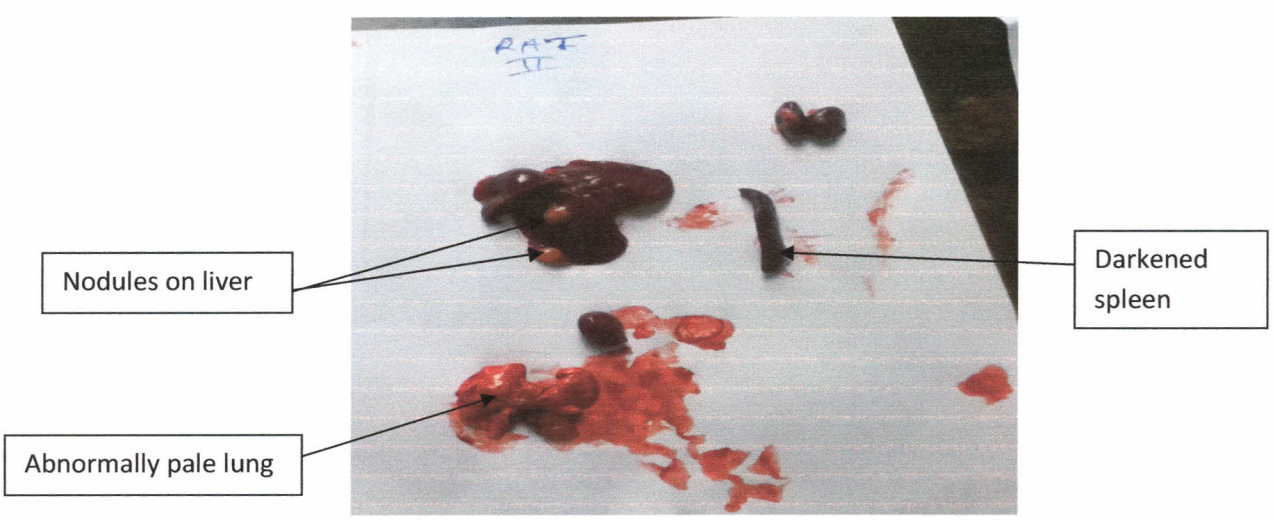
Test rats on BZ 013

Figure 11; Photographs showing morphological changes on rat 1,2,3 and 4.

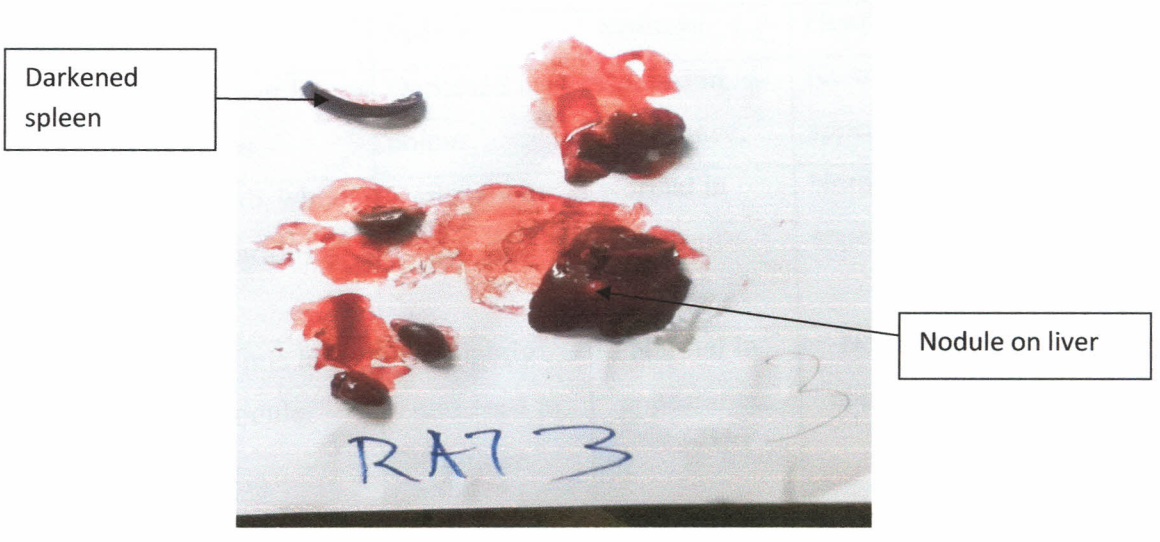
Rat 1



Rat 2



Rat 3


Darkened spleen
Nodule on liver
RAT 3

Rat 4

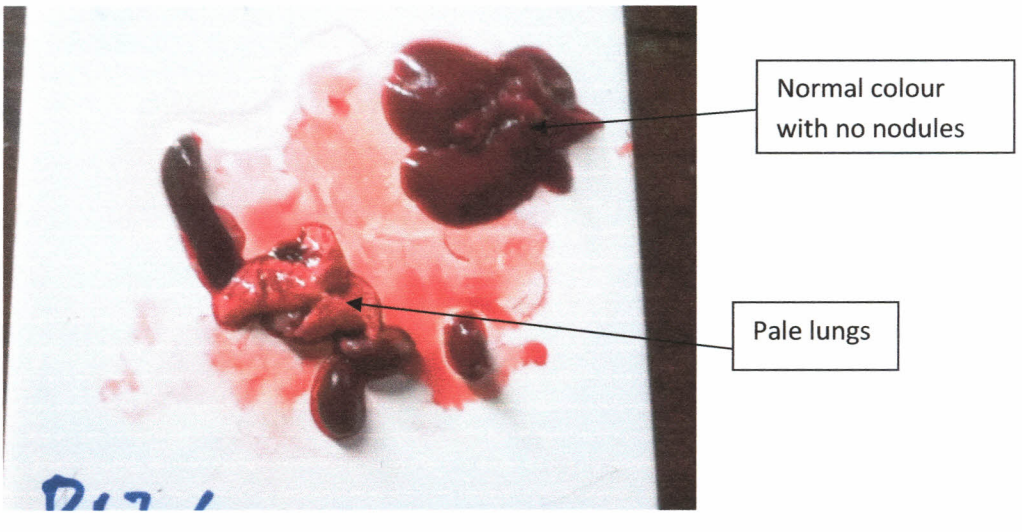

Normal colour with no nodules
Pale lungs

Table 10: Morphological changes observed on the various internal organs for the test rats

Rats	**Organ morphology**				
	Liver	**Spleen**	**Kidneys**	**Heart**	**Lungs**
R1	Presence of four large white nodules.	Darkened in colour.	Normal in appearance.	Normal in appearance.	Abnormally pale in colour.
R2	Presence of six white nodules of various sizes.	Darkened in colour	Normal in appearance.	Normal in appearance.	Very pale in colour.
R3	Presence of one large white nodule.	Slightly darkened in colour.	Normal in appearance.	Normal in appearance.	Very pale with a mottled appearance.
R4	Normal in colour with no nodules.	Slightly darkened in colour.	Normal in appearance.	Normal in appearance.	Mottled in appearance.

3.6 Biochemical markers of liver and kidney function

Table 11: Rat Biochemical Reference Ranges [19,20]

Total protein	5.6-7.6 g/dL	Albumin	3.8-4.8 g/dL
Glucose	50-135 mg/dL	BUN	15-21 mg/dL
Creatinine	0.2-0.8 mg/dL	Sodium	143-156 mEq/L
Potassium	5.4-7 mEq/L	Chloride	100-110 mEq/L
Phosphorous	3.11-11 mg/dL	Calcium	5.3-13 mg/dL
ALT	17.5-30.2 U/L	AST	45.7-80.8 U/L
Alkaline phos	56.8-128 U/L	Cholesterol	40-130 mg/dL
Total bilirubin	0.2-0.55 mg/dL	Amylase	128-313 SU/dL
BUN = blood urea nitrogen		ALT = alanine aminotransferase	
AST = aspartate aminotransferase			

Table 12: Results of biochemical markers from BZ 013

NO.	SAMPLE ID	SGPT (U/L)	SGOT (U/L)	UREA (mmol/L)	CREAT (umol/L)
1	BZ 013 MBURU 01	273	655	11	55
2	BZ 013 MBURU 02	288	538	13	70
3	BZ 013 MBURU 03	*	728	15	94
4	BZ 013 MBURU 04	284	#	12	69
AVERAGE		282	640	12.3	72

Note;

* Indicates no value was obtained due to insufficient sample volume.

Indicated no reading was obtained even after repeated measurement of the sample.

Table 13: Results of biochemical markers from the positive controls

NO.	SAMPLE ID	SGPT (U/L)	SGOT (U/L)	UREA (mmol/L)	CREAT (umol/L)
1	CARBON TET 01	232	173	13	82
2	CARBON TET 02	127	216	11	128
3	CARBON TET 03	382	551	16	80
AVERAGE		237	313	13	97

Table 14: Results of biochemical markers from the negative controls (Untreated)

NO.	SAMPLE ID	SGPT (U/L)	SGOT (U/L)	UREA (mmol/L)	CREAT (umol/L)
1	NORMAL 01	31	#	6.9	55
2	NORMAL 02	97	12	5.8	48
3	NORMAL 03	29	40	6.3	59
AVERAGE		62	26	6.3	54

Deductions on Liver Toxicity

An elevation of liver transaminases is a sign of liver toxicity.[21,22,23] From the tabulated results above, these enzymes, namely SGOT and SGPT, can be seen to be markedly elevated from the expected results in the former table.

In the animals under test with BZ 013, it can be observed that the average levels of SGPT stand at 282 U/L, which is about twelve times the expected range of 17.5-30.2 U/L (Rat ref. ranges)

The average levels of SGOT stand at 640 U/L, which is about ten times the expected range of 45.7-80.8 U/L. This is an indication that the test substance actually did cause severe liver toxicity in the test animals.

From the tabulated results of the positive and negative controls above, it can be observed that the levels of the transaminases in the negative controls are much lower than in the positive controls. The levels of the SGPT (ALT) in the positive controls is 237 U/L as compared to the 62U/L in the negative controls. Both values are above the reference ranges for the rats with the positive controls about five times more and the negatives being about two times more. This indicates that there was significant liver toxicity in the positive control and a suspected toxicity in the negative control. The levels of SGOT (AST) for the positive controls (313 U/L) are also higher than those of the negative controls (26 U/L). The interpretation of this is that there was significant toxicity in the positive controls but not in the negative controls.

This is an indication that the dose of the test substance BZ 013 given was very toxic to the animal and thus caused significant liver toxicity.

Deductions on Renal Toxicity

Using the SI Unit Conversion Calculator (see appendix), the rat reference ranges are 5.355-7.497 mmol/L (factor 0.357) for the blood urea nitrogen and 15.232-61.008 umol/L (factor 76.26) for the creatinine.[23]

The average urea levels for BZ 013 are 12.3 mmol/L, which is higher than the expected rat ref. ranges as indicated above. This indicates an accumulation of urea in blood which is a sign of

compromised renal function. Thus it can be deduced that the test substance *BZ 013* did actually cause significant renal toxicity.

The average blood urea levels for the positive controls on Carbon tetrachloride was 13mmol/L, which is also above the ref. range above. Thus the CCl_4also did cause significant renal toxicity. The average blood urea levels for the untreated animals was 6.3 mmol/L which is well within the range thus no toxicity was observed in this case.

The creatinine levels for the test rats was 72 umol/L while those for the positive control was 97 umol/L. This indicates the accumulation of creatinine in blood in both cases above the levels expected. Thus there was toxicity in both. This is in contrast with the negative control,54 umol/L which is well within the range provided. Thus there was no toxicity observed in the latter.

Figure 12; A comparative bar graph showing average liver transaminase levels

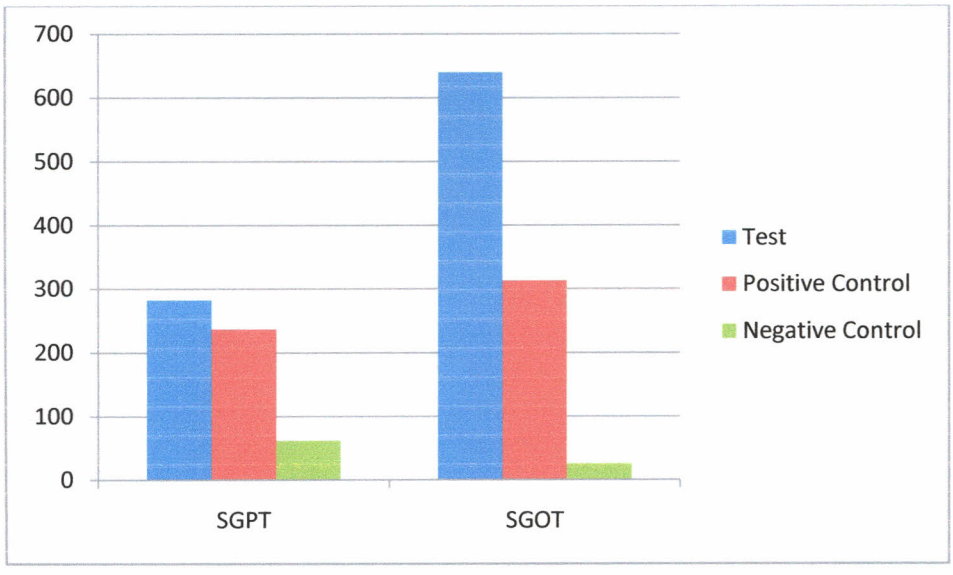
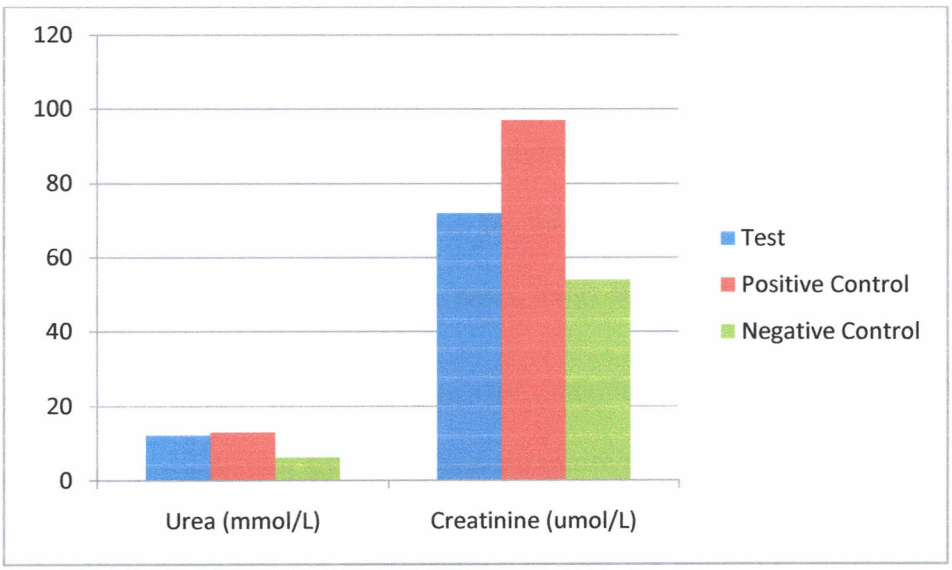


Figure 13; A comparative bar graph showing average liver transaminase levels



4.0 CHAPTER 4: CONCLUSIONS AND RECCOMENDATIONS

Conclusion

The test product *BZ 013* when taken at higher doses than recommended causes significant toxicity to the body. It not only affects the liver and kidney function but also significantly affects other major body organs, namely the heart, lungs and spleen.

This therefore implies that the drug has the potential to cause severe cardiotoxic episodes, possibility of respiration failure or even compromised immune function.

It should be noted that this information regarding the toxicity profile of the drug is not provided either in an insert or on the website for the drug.

The clinical trials as pertaining to this drug also involved only 31 individuals which is obviously not adequate. This study also involved only four rats which is not adequate for solid conclusions to be made on the results.

Recommendations More information as pertaining to the safety profile of this product, *BZ 013,* should be availed by the manufacturers to the general public. A safe dose for children should also be provided as the website only provides only adult doses, which could lead to wrong dose estimation thus a drug overdose is possible.

Consumers of this product and other herbal products should also make an attempt to know their potential side effects so that they can make informed decisions especially when choosing drugs for chronic use, like *BZ 013,* which is used for 'treatment' of HIV/AIDS.

4.1 References

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23. SBDR - SOCIETY FOR BIOMEDICAL DIABETES RESEARCH,SI Unit Conversion Calculator.

4.2 Appendices

SBDR - SOCIETY FOR BIOMEDICAL DIABETES RESEARCH; SI Unit Conversion Calculator [23]

Conversion table for chemical compounds from conventional to SI units

Conventional unit => SI unit: multiply by factor

SI unit => conventional unit: divide by factor

Table 15: ; SI Unit Conversion Calculator

Compound	Conventional (US) Unit	Factor	SI Unit
Alanine aminotransferase (ALT)	units/l	1.0	U/L
Aspartate aminotransferase (AST)	units/l	1.0	U/L
Creatine	mg/dl	76.26	µmol/l
Urea nitrogen	mg/dl	0.357	mmol/l