ISOLATION, CHARACTERIZATION AND PATHOLOGIC STUDIES OF AFLATOXIN AND *ASPERGILLUS* SPECIES FROM LOCAL CASHEW NUTS CONSUMED IN ENUGU STATE, NIGERIA

BY

MBEGBU, OBIAGELI DORIS
PG/M.SC/13/65398

DEPARTMENT OF MEDICAL LABORATORY SCIENCES
FACULTY OF HEALTH SCIENCES AND TECHNOLOGY
COLLEGE OF MEDICINE
UNIVERSITY OF NIGERIA
ENUGU CAMPUS

MAY 2016

ISOLATION, CHARACTERIZATION AND PATHOLOGIC STUDIES OF AFLATOXIN AND *ASPERGILLUS* SPECIES FROM LOCAL CASHEW NUTS CONSUMED IN ENUGU STATE, NIGERIA

BY

MBEGBU, OBIAGELI DORIS
PG/M.SC/13/65398

AREA OF MAJOR SPECIALITY: MICROBIOLOGY

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES

FACULTY OF HEALTH SCIENCES AND TECHNOLOGY
COLLEGE OF MEDICINE
UNIVERSITY OF NIGERIA
ENUGU CAMPUS

SUPERVISOR: PROF. N.F. ONYEMELUKWE

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS DEGREE IN MEDICAL LABORATORY SCIENCES

MAY 2016

DEDICATION

This work is dedicated to God Almighty and our savior Jesus Christ

ACKNOWLEDGMENT

This is to express my profound gratitude to God Almighty for making this work a success. My sincere gratitude goes to my supervisor, Professor N.F. Onyemelukwe whose direction, understanding, patience, generosity, humility, encouragement, supervisory competence and motherly love led to the successful completion of this work.

I am grateful to the Head of Department, Professor N.F. Onyemelukwe and all the lecturers especially Prof. E.O Ukaejiofor, Barrister P.U. Achukwu, Dr. C.N. Okwuosa, Dr. T.K.C Udeani, Dr. E.A Onyebueke, Dr. S. Ufelle, Dr O. Dozie-Nwakile, who contributed in so many ways to impart knowledge to me. Special thanks to Dr Azubike for her assistance and professional advice throughout this work. My profound gratitude goes to my mother, Mrs. Jully Mbegbu for her motherly advice, prayers and financial support, just to see that I succeeded in life. To my siblings, Okechukwu, Eby, Chinenye, Chinedu and kenechukwu, I say thank you for your financial supports and fervent prayers during the course of this work and my final exams. I love you all and will always do my best to make you proud. To my nephews; Ikenna, T. boy, Chinweike, Kenenna, James, Miracle and my nieces: Chi-Chi, Amanda, Muna and Uchee, I appreciate you all. I recognize my pastor, Barrister Edwin Biayeibo for his prayers and mentorship.

Finally, my unalloyed thanks go to my project mates, medical students, non-medical students and all who contributed immensely to the success of this work.

ABSTRACT

In a study conducted to investigate the isolation, characterization, pathologic studies of aflatoxin and *Aspergillus* species from local cashew nuts, a total of 152 samples of exposed and unexposed roasted cashew nuts were analyzed between May and December 2015. A total of six genera of fungal isolates consisting of *Aspergillus* species 178*(76.0%*), *Mucor* species 23(9.8%), *Rhizopus* species 17(7.3%), *Rhodotorula* species 11(4.7%), *Candida* species *4(*1.7%), and *Geotrichum* species 1(.04%) being the least were isolated. Out of the 62 samples of exposed cashew nuts group, *Aspergillus niger* showed the highest incidence 32(37.2%) followed by *Aspergillus flavus* 22(25.5%), *Mucor* species 12(13.9%), *Aspergillus fumigatus* 8(9.3%), *Rhizopus* species 7(8.1%), *Rhodotorula* species 3(3.4%), *Aspergillus nidulans* 1(1.1%) and *Candida* species 1(1.1%). *Geotrichum* species was not isolated from this group. Of the 90 samples of cashew nuts under the unexposed category, *Aspergillus flavus* ranked highest 61(41.2%) followed by *Aspergillus niger* 49(33.1%), *Mucor* species 11(7.4%), *Rhizopus* species 10(6.7%), *Rhodotorula* species 8(5.4%), *Aspergillus fumigatus 4(2.7%), Candida* species *3(2.0%), Aspergillus nidulans 1(0.6%) and Geotrichum* species *1*(0.6%). The total fungal isolates from unexposed cashew nuts (148) were greater in number than the fungal isolates from the exposed group (86) whose difference was not statistically different (P>0.05). Out of 62 samples exposed cashew nuts investigated, *Aspergillus flavus* yielded mixed growth with other fungal isolates. *Aspergillus niger* + *Aspergillus flavus 9* (64.2%) showed the highest incidence while *Aspergillus flavus* + *Aspergillus fumigatus 1*(14.2%) showed the least growth. Of the 90 samples of unexposed cashew nuts investigated, *Aspergillus flavus* + *Aspergillus niger* 29(63%) showed the highest incidence while *Aspergillus flavus* + *Aspergillus nidulans 1(2.1%)* and *Aspergillus flavus* + *Aspergillus fumigatus 1 (2.1%*) showed the least incidence. However there was a significant difference observed between the mixed growth of *Aspergillus flavus* + *Aspergillus nidulans* and *Aspergillus flavus* + *Geotricum* species (P < 0.05). Of the 234 fungal isolates encountered in the study, 83(35.4%) of *Aspergillus flavus* were isolated. Similarly, there was a significant difference between the number of *Aspergillus flavus* and *non- Aspergillus flavus* isolated. (P< 0.05). Out of the 83(35.4%) of the *Aspergillus flavus*-contaminated cashew nuts examined, 17(65.3%) yielded positive aflatoxin detection (P> 0.05). In the study conducted to investigate the pathological effects of the consumption of contaminated cashew nut using wistar rats fed with a 50% aflatoxin contaminated cashew nut diet, the liver and renal function test results showed no statistically significant difference (P>0.05) when the test and control animals were compared, though the calcium Ca^{2+} and creatinine levels showed a statistically significant difference (P<0.05). The histological results however revealed some widening of the sinusoids, central canal rupture, degenerating hepatocytes and inflammatory cellular infiltration especially within the sinusoids suggesting cirrhosis for the test animals while the kidney shows normal features. The result of the study suggests that the consumption of contaminated cashew nuts which is a major snack food widely patronized in Enugu may pose a serious public health hazard that may target the liver aside other minor issues. This therefore emphasizes the pertinent need for cashew nut protection studies and implementation in line with the recommendation of the FAO (Food and Agriculture Organization) and WHO (World Health Organization).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATE

CHAPTER ONE

2.0 INTRODUCTION

The cashew plant, *Anacardium occidentale,* is a small to medium size tree belonging to the family *Anacardiaceace*. (Koehler, 1887). It is a tropical evergreen tree that produces the cashew seed and the cashew apple. The cashew apple is a kidney-shaped achene about 3cm long with a hard grey-green pericarp. The seeds are the source of cashew nuts. The cashew tree can grow as high as 14 meters (46 ft), but the dwarf cashew, growing up to 6 meters (20 ft), has proved more profitable, with earlier maturity and higher yields (Cobley and steele, 1976).

Scientific classification of cashew:

Kingdom: *Plantae*

Order : *Sapindales*

Family : *Anacardiaceae*

Genus : *Anarcardium*

Species : *Anarcardium occidentale* (Wikipedia, 2014).

The cashew seed, often simply called a cashew, is widely consumed. It is eaten on its own, used in recipes, or processed into cashew cheese or cashew butter. The shell of the cashew seed yields derivatives that can be used in many applications from lubricants to paints, and other parts of the tree have traditionally been used for snake-bites and other folk remedies. Originally native to north-eastern Brazil, the tree is now widely cultivated in Vietnam, Nigeria and India as major production countries (Rosen *et al*., 1994). In Nigeria however, despite the cultivation of cashew in lantations and establishment of cashew-processing factories (Esuruoso, 1974), peasant processing and packaging methods are still commonly adopted.

õThe cashew tree is large and evergreen, growing to 10-12 m (-32 ft) tall, with a short, often irregularly shaped trunk. The leaves are spirally arranged, leathery textured, elliptic to obovate, 4 to 22 cm long and 2 to 15 cm broad, with smooth margins. The largest cashew tree in the world covers an area of about 7,500 square meters (81,000 sq ft); it is located in Natal, Rio Grande do Norte, Brazil (Rosen *et al*., 1994).

The fruit of the cashew tree is an accessory fruit which is an oval or pear-shaped structure, a hypocarpium, that develops from the pedicel and the receptacle of the cashew flower called the cashew apple, better known in Central America as maranon, it ripens into a yellow and/or red structure about 5-11 cm long. It is edible, and has a strong ñsweetò smell and a sweet taste. The pulp of the cashew apple is very juicy, but the skin is fragile, making it unsuitable for transport. In Latin America, a fruit drink is made from the cashew apple pulp which has a very refreshing taste and tropical flavor that can be described as having notes of mango, raw green pepper, and just a little hint of grapefruit-like citrus. Some people are allergic to cashews, but cashews are a less frequent allergen than tree nuts or peanuts. The delicately flavored cashew nut is a favorite between meal snacks that can be readily found in your local market year round. It also makes wonderful nut butter and a special addition to salads and stir-fry dishes.

Cashew nuts are actually the kidney-shaped seeds that adhere to the bottom of the cashew apple, the fruit of the cashew tree, which is native to the coastal areas of north-eastern Brazil.

Fungi are members of large group of eukaryotic organisms that include micro- organism such as yeast, moulds as well as mushrooms. They are non motile with a range of internal membrane systems, membrane bound organelles and a well defined cell wall composed largely of polysaccharides such as Glucan, Mannan and Chitin (Warnock, 2007). Some are yeast which are

round, oval or elongated unicellular organism forming moist or mucoid colonies. Others are moulds which form mycelia which may be partitioned by septa or cross wall.

Aspergillus is a member of the Deuteromycetes fungi, which is a group with no known sexual state. With DNA evidence forthcoming, it is likely that all members of the genus *Aspergillus* are closely related and should be considered members of the Ascomycota (Wikipedia, 2014). While species vary in color, size and growth rate, microscopic characteristics are fairly uniform across *Aspergillus species*. For example, all have hyphae that are septate and hyaline. Hyphae and conidia are separate (MicrobeWiki, 2014). Members of the genus posses the ability to grow where there is a high osmotic concentration (high sugar, salt, etc). *Aspergillus species* are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Commonly, fungi grow on carbon-rich substrate like monosaccharides (such as glucose) and polysaccharides (such as amylase) (Wikipedia, 2014).

CLASSIFICATION

Kingdom: fungi

Phylum: *Ascomycota*

Class: *Eurotiomycetes*

Order: *Eurotiales*

Family: *Trichocomacaeae* (Geiser, 2009).

Aflatoxins constitute a family of toxic metabolites with low molecular weight produced by some strains of *Aspergillus flavus and* related organisms including *Aspergllus parasiticus, Aspergillus*

ruber, Aspergillus nominus and Aspergillus tamari (Bhat *et al.,* 2003; Leitao *et al*.,1989; Goto *et al.,* 1996). Aflatoxin is about the most popular and widespread mycotoxin (Agrios, 1978). They are recognized to be hepatotoxins and carcinogenes for humans (WHO, 1998). The primary metabolites of *Aspergillus flavus* are aflatoxins B1, B2 G1 and G2 which contaminate a variety of agricultural food and feed products (Diener *et al.,*in Blatt (Ed.) 1996). *Aspergillus* and *Penicillium* produce their toxins mostly in stored seeds, hay or commercially processed foods and feed although infection of seeds usually take place in the field (Amadi *et al*., 2009). Several disease outbreaks of aflatoxicosis in humans and animals have been reported due to the consumption of aflatoxin contaminated food and feed (Reddy and Raghavender, 2007).

However, mycotoxicoses are becoming increasingly implicated in human and animal pathology (Bacha *et al.,* 1988).

Other group of diseases caused by *Aspergillus* is Aspergillosis. The most common subtype among paranasal sinus infections associated with aspergillosis ia *Aspergillus fumigatus* (Bozkurt *et al.,* 2008). The symptoms include fever, cough, chest pain. In humans, the major forms of disease are:

- Allergic bronchopulmonary aspergillosis
- Acute invasive aspergillosis
- Disseminated invasive aspergillosis
- Aspergilloma.

  (Wikipedia, 2014).

2.1 AIMS AND OBJECTIVES

- The aim of this study is to isolate, characterize and study the pathologic effects of aflatoxins on liver and kidney of albino wistar rats.

Specific Objectives

- Screen cashew nuts sold in Enugu metropolis for evidence of fungal contamination.
- Categorize the *Aspergillus species* from exposed and unexposed cashew nuts.
- Screen the cashew nuts harboring *Aspergillus flavus* isolates for evidence of aflatoxin contamination.
- Assess any pathological effects in albino wistar rats fed with aflatoxin contaminated cashew nuts after 10 days of feeding.
- Determine the electrolyte, urea, creatinine and liver functions test values of the wistar rats after 10 days of feeding.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

2.1.1 HISTORY OF CASHEW TREE

The cashew tree is native to coastal areas of Brazil although it is now widely cultivated in Vietnam, Nigeria, and India as major production countries (FAO, 2013). In the 16th century, Portuguese explorers took cashew trees from this South American country and introduced them into other tropical regions such as India and some African countries, where they are now also cultivated (*Cajucultura historia,* 2010). The cashew tree has always been a prized resource owing to its precious wood, cashew balm and cashew apple, but the cashew nut itself did not gain popularity until the beginning of the 20th century. Today, the leading commercial producers of cashews are India, Brazil, Mozambique, Tanzania and Nigeria.

Its English name derives from the Portuguese for the fruit of the cashew tree caju (Portuguese pronunciation: [ke Zu]), which itself is derived from the indigenous Tupian name *acaju,* literally meaning ñnut that produces itself (Embrapa, 2012). The name *Anacardium,* originally from the Greek, refers to the unusual location of the seed outside the core or heart of the fruit (ana means ñwithoutò and ï cardium means ñheartò).

The cashew plant, *Anacardium occidentale,*is a small to medium size tree belonging to the family *Anacardiaceace*. (Koehler 1887). It is a tropical evergreen tree that produces the cashew seed and the cashew apple. The cashew apple is a kidney-shaped achene about 3cm long with a hard grey-green pericarp. The seeds are the source of cashew nuts. The cashew tree can grow as high as 14 meters (46 ft), but the dwarf cashew, growing up to 6 meters (20 ft), has proved more profitable, with earlier maturity and higher yields.

The cashew seed, often simply called a cashew, is widely consumed. It is eaten on its own, used in recipes, or processed into cashew cheese or cashew butter. The cashew apple is light reddish to yellow fruit, whose pulp can be processed into a sweet, astringent fruit drink or distilled into liquor.

The shell of the cashew seed yields derivatives that can be used in many applications from lubricants to paints, and other parts of the tree have traditionally been used for snake-bites and other folk remedies.

Originally native to north-eastern Brazil, the tree is now widely cultivated in Vietnam, Nigeria and India as major production countries (Rosen *et al*., 1994).

2.1.2 HABITAT AND GROWTH

óAnacardium occidentaleô, from Koehierôs óMedicinal-Plantsô (1887). The cashew tree is large and evergreen, growing to 10-12 m (-32 ft) tall, with a short, often irregularly shaped trunk. The leaves are spirally arranged, leathery textured, elliptic to obovate, 4 to 22 cm long and 2 to 15 cm broad, with smooth margins. The flowers are produced in a panicle or corymb up to 26 cm long; each flower is small, pale green at first, then turning reddish, with five slender, acute petals 7 to 15 mm long. The largest cashew tree in the world covers an area of about 7,500 square meters (81,000 sq ft); it is located in Natal, Rio Grande do Norte, Brazil.

Plate 2.1: The Cashew tree

2.1.3 THE CASHEW FRUIT

The fruit of the cashew tree is an accessory fruit (sometimes called a pseudocarp or false fruit). What appears to be the fruit is an oval or pear-shaped structure, a hypocarpium, that develops from the pedicel and the receptacle of the cashew flower Called the cashew apple, better known in Central America as maranon, it ripens into a yellow and/or red structure about 5-11 cm long. It is edible, and has a strong ñsweetò smell and a sweet taste (Varghese *et al.,* 1964). The pulp of the cashew apple is very juicy, but the skin is fragile, making it unsuitable for transport. In Latin America, a fruit drink is made from the cashew apple pulp which has a very refreshing taste and tropical flavor that can be described as having notes of mango, raw green pepper, and just a little hint of grapefruit-like citrus.

Cashew nuts are more popular than cashew apples in many parts of the world that do not grow cashews, because the fruit, unlike the nut, is difficult to transport (Bavier, 2014). Cashew apple juice, however, may be used for manufacturing blended juices. Cashew apples have a sweet but astrigebnt taste traced to the waxy layer on the skin that contains a chemical,urushiol, which can cause minor skin irritation to areas that have had contact with it (Harold, 2004), (BBC, 2013).

Plate 2.2: The Cashew Apple

2.1.4 CASHEW SEED

The true fruit of the cashew tree is a kidney or boxing-glove shaped drupe that grows at the end of the cashew apple. The drupe develops first on the tree, and then the pedicel expands to become the cashew apple. Within the true fruit is a single seed which is often considered a nut in the culinary sense. The seed is surrounded by a double shell containing an allergenic phenolic resin, anacardic acid, a potent skin irritant chemically related to the better-known allergenic oil urushiol which is also a toxin found in the related poison ivy. Properly roasting cashews destroys the toxin, but it must be done outdoors as the smoke (not unlike that from burning poison ivy) contains urushiol droplets which can cause severe, sometimes life-threatening, reactions by irritating the lungs. People who are allergic to cashew (or poison ivy), urushiol may cross-react to mango or pistachio which are also in the Anacardiaceae family. Some people are allergic to cashews, but cashews are a less frequent allergen than tree nuts or peanuts (Rosen *et al.,* 1994) While the cashew plant is native to northeast Brazil, the Portuguese took it to Goa, India, between 1560 and 1565 (*Cajucultura historia,* 2010).

2.1.5 CASHEW NUT

The delicately flavored cashew nut is a favorite between meal snack that can be readily found in your local market year round. It also makes wonderful nut butter and a special addition to salads and stir-fry dishes.

Cashew nuts are actually the kidney-shaped seeds that adhere to the bottom of the cashew apple, the fruit of the cashew tree, which is native to the coastal areas of northeastern Brazil. While cashew apples are not appreciated in the United States, they are regarded as delicacies in Brazil

and the Caribbean. Cashews are always sold shelled because the interior of the shells contains a caustic resin, known as cashew balm, which must be carefully removed before the nuts are fit for consumption. This caustic resin is actually used in industry to make varnishes and insecticides.

Plate 2.3: Cashew nuts tied in hand –knotted polyethylene bag

2.1.6 CASHEWS AS A SNACK

Botanically speaking, cashews are not actually nuts but merely seeds. Culinary uses for cashew seeds are similar to uses for nuts, however, and the seeds are frequently referred to as nuts. Cashews, unlike oily tree nuts, contain starch to about 10% of their weight. This makes them more effective than nuts in thickening water-based dishes such as soups, meat stews, and some Indian milk-based desserts. Many Southeast Asian cuisines use cashews for this unusual characteristic, rather than other nuts (Harold, 2004). The shell of the cashew nut is toxic, which is why the nut is never sold in the shell to consumers

Cashew nuts are commonly used in Indian cuisine, whole for garnishing sweets or curries, or ground into a paste that forms a base of sauces for curries (e.g., korma), or some sweets (e.g*., kafu barfi).* It is also used in powdered form in the preparation of several Indian sweets and desserts. In *Goan cuisine*, both roasted and raw kernels are used whole for making curries and sweets.

The cashew nuts can be harvested in its tender form, when the shell has not hardened and is green in colour. The shell is soft and can be cut with a knife and the kernel extracted, but it is already corrosive at this stage, so gloves are required. The kernel can be soaked in turmeric water to get rid of the corrosive material before use. Cashew nuts are also used in *Thai* and Chinese cuisine, generally in whole form.In Philipines, cashew is a known product of Antipolo, and is eaten with *suman. In Indonesia,* roasted and salted cashew nuts is called *kacang mete or kacang mede* while the cashew apple is called *jambu monyet.*

In Mozambique*, bolo polana* is a cake prepared using powdered cashews and mashed potatoes as the main ingredients (Phillippa, 2009).

South American countries have developed their own specialties. In Brazil, the cashew fruit juice is popular all across the country. In Panama, the cashew fruit is cooked with water and sugar for a prolonged time to make a sweet, brown, paste-like dessert called *dulce de maranon.*

2.1.7 HEALTH BENEFITS OF CASHEW NUTS

- **Heart-Protective Monounsaturated Fats**

Not only do cashews have a lower fat content than most other nuts, approximately 82% of their fat is unsaturated fatty acids, plus about 66% of this unsaturated fatty acid content are heart-healthy monounsaturated fats, similar to those found in olive oil. Studies of diabetic patients show that monounsaturated fat, when added to a low-fat diet, can help to reduce high triglyceride levels. Triglycerides are a form in which fats are carried in the blood, and high triglyceride levels are associated with an increased risk for heart disease, so ensuring you have some monounsaturated fats in your diet by enjoying cashews is a good idea, especially for persons with diabetes (Resnicow *et al.,* 1991).

- **Cardio-protective benefits**

Research published in the British Journal of Nutrition identified several nuts among plant foods with the highest total antioxidant content, suggests nutôs high antioxidant content may be key to their cardio-protective benefits (Blomhoff *et al.*,2006).

Nutsô high antioxidant content helps explain results seen in the Iowa Womenôs Health Study in which risk of death from cardiovascular and coronary heart diseases showed strong and consistent reductions with increasing nut/peanut butter consumption. Total death rates decreased 11% and 19% for nut/peanut butter intake once per week and 1-4 times per week, respectively.

Even more impressive were the results of a review study of the evidence linking nuts and lower risk of coronary heart disease, also published in the British Journal of Nutrition. (Kelly *et al*., 2006) In this study, researchers looked at four large prospective epidemiological studiesð the Adventist Health Study, Iowa Womenôs Study, NursesôHealth Study and the Physicianôs Health Study. When evidence from all four studies was combined, subjects consuming nuts at least 4 times a week showed a 37% reduced risk of coronary heart disease compared to those who never or seldom ate nuts. Each additional serving of nuts per week was associated with an average 8.3% reduced risk of coronary heart disease (Hu *et al.,* 1999).

- **Copper for antioxidant defenses, energy production, bones and blood vessels**

An essential component of many enzymes, plays a role in a wide range of physiological processes including iron utilization, elimination of free radicals, development of bone and connective tissue, and the production of the skin and hair pigment called melanin (Blomhoff *et al.*, 2006). For example, copper is an essential component of the enzyme, superoxide dismutase, which is important in energy production and antioxidant defenses.

Copper is also necessary for the activity of *lysyl oxidase,* an enzyme involved in cross- linking collagen and elastin, both of which provide the ground substance and flexibility in blood vessels, bones and joints. Low dietary intake of copper may also be associated with increased fecal free radical production and fecal water alkaline phosphatase activity, risk factors for colon cancer (Davis, 2003).

Numerous health problems can develop when copper intake is inadequate, including iron deficiency anemia ruptured blood vessels, osteoporosis, joint problems such as rheumatoid arthritis, brain disturbances, elevated LDL (bad) cholesterol and reduced HDL (good) cholesterol levels (Tsai *et al.*, 2004), irregular heartbeat, and increased susceptibility to infections.

- **Cashews help supply calcium and magnesium for strong bones**

Everyone knows that calcium is necessary for strong bones, but magnesium is also vital for healthy bones. About two-thirds of the magnesium in the human body is found in our bones. Some helps give bones their physical structure, while the rest is found on the surface of the bone where it is stored for the body to draw upon as needed. Magnesium, by balancing calcium, helps regulate nerve and muscle tone. In many nerve cells, magnesium serves as Natureôs own calcium channel blocker, preventing calcium from rushing into the nerve cell and activating the nerve. By blocking calciumôs entry, magnesium keeps our nerves relaxed. If our diet provides us with too little magnesium, however, calcium can gain free entry, and the nerve cell can become over activated, sending too many messages and causing excessive contraction.

Insufficient magnesium can thus contribute to high blood pressure, muscle spasms (including spasms of the heart muscle or the spasms of the airways symptomatic of asthma). And migraine headaches, as well as muscle cramps, tension, soreness and fatigue. Given these effects, it is not surprising that studies have shown magnesium helps reduce the frequency of migraine attacks, lowers blood pressure, helps prevent heart attacks, promotes normal sleep patterns in women suffering from menopausal sleep disturbances, and reduces the severity of asthma.

- **Cashews help prevent gallstones**

Twenty years of dietary data collected on 80,000 women from the Nursesô Health Study shows that women who eat least 1 ounce of nuts, peanuts or peanut butter each week have a 25% lower risk of developing gallstones. Since 1 ounce is only 28.6 nuts or about 2 tablespoons of nut butter, preventing gallbladder disease may be as easy as packing one cashew butter and jelly sandwich (be sure to use whole wheat bread for its fiber, vitamins and minerals) for lunch each

week, having a handful of cashews as an afternoon pick me up, or tossing some cashews on your oatmeal or salad.

- **Eating nuts lowers risk of weight gain**

Although nuts are known to provide a variety of cardio-protective benefits, many avoid them for fear of weight gain. A prospective study published in the journal Obesity shows such fears are groundless. In fact, people who eat nuts at least twice a week are much less likely to gain weight than those who almost never eat nuts (Bes-Rastrollo *et al.,* 2007).The 28-month study involving 8,865 adult men and women in Spain, found that participants who ate nuts at least two times per week were 31% less likely to gain weight than were participants who never or almost never ate nuts.

And, among the study participants who gained weight, those who never or almost never ate nuts gained more (an average of 424 g more) than those who ate nuts at least twice weekly.

Study authors concluded, ñFrequent nut consumption was associated with a reduced risk of weight gain (5kg or more). These results support the recommendation of nut consumption as an important component of a cardio-protective diet and also allay fears of possible weight gain (Bes-Rastrollo *et al*., 2007).

2.1.8 DESCRIPTION OF CASHEW NUTS

Cashew nuts are actually seeds that adhere to the bottom of the cashew apple, the fruit of the cashew tree, which is native to the coastal areas of northeastern Brazil. Cashew apples, while not known in the U.S., are regarded as delicacies in Brazil and the Carribean. The seed we know as the kidney-shaped cashew ñnutò is delicate in flavor and firm, but slightly spongy, in texture (https;//en.wiktionary.org).

You have probably noticed that cashews in the shell are not available in stores. This is because these nuts are always sold pre-shelled since the interior of their shells contains a caustic resin, known as cashew balm, which must be carefully removed before they are fit for consumption. This caustic resin is actually used in industry to make varnishes and insecticides. Cashews, known scientifically as *Anacardium occidentale,* belong to the same family as the mango and pistachio nut (Koehler 1887).

2.1.9 NUTRITIONAL VALUE OF CASHEW NUTS

Nutritional value per 100 g (3.5 oz)

Energy	553 kcal (2,310 kJ)
Carbohydrates	30.19 g
Starch	0.74 g
Sugars	5.91 g
Lactose	0.00g
Dietary fiber	3.3 g
Fat	43.85 g
Saturated	7.783 g
Monounsaturated	23.797g
Polyunsaturated	7.845 g
Protein	18.22 g

VITAMINS

Vitamin A	0 IU
Thiamine (Bi)	0.423mg
Riboflavin (B2)	0.058mg

Niacin (B3)	1.062mg
Pantothenic acid (B5)	0.86mg
Vitamin B6	0.417mg
Folate (B9)	25µg
Vitamin B 12	0µg
Vitamin C	0.5mg
Vitamin D	0µg
Vitamin E	0.90mg
Vitamin K	34.1µg

MINERALS

Calcium	37mg
Iron	6.68mg
Magnesium	292mg
Manganese	1.66mg
Phosphorus	593 mg
Potassium	660 mg
Sodium	12mg
Zinc	5.78mg

OTHER CONSTITUENTS

Water	5.20g

In a 100 gram serving, raw cashews provide 553 calories, 67% of the Daily Value (DV) in total fats, 36% DV of protein, 13% DV of dietary fiber and 11% DV of carbohydrates (table).U41

Cashews are rich sources (> 19% DV) of dietary minerals, including particularly copper, manganese, phosphorus and magnesium (79-110% DV), and of thiamin, vitamin B6 and vitamin K (32-37% DV). (https;//en.wiktionary.org).

2.1.10 PRODUCTION OF CASHEW NUTS

Top 5 Countries for Production of Cashew Nuts (with shell) in 2013 (https;//en.wiktionary.org).

Country	**Production MT (metric tons)**
Vietnam	1,110,800
Nigeria	950,000
India	753,000
Cöte dôIvoire	450,000
Benin	180,000
World Total	**4,439,960**

The cashew nut can also be harvested in its tender form, when the shell has not hardened and is green in color. The shell is soft and can be cut with a knife and the kernel extracted, but it is already corrosive at this stage, so gloves are required. The kernel can be soaked in turmeric water to get rid of the corrosive material before use. Cashew nuts are also used in Iii and Chinese cuisine, generally in whole form.

In the Philippines, cashew is a known product of *Antipolo,* and is eaten with *suman.* Pampanga also has a sweet dessert called *turrones de casuy*, which is cashew marzipan wrapped in white wafers.

In Indonesia, roasted and salted cashew nut is called *kacang mete or kacang mede*, while the cashew apple is called *jambu monyet* (literally means monkey rose apple).

In Mozambigue*, bolo polana* is a cake prepared using powdered cashews and mashed potatoes as the main ingredients. This dessert is popular in South Africa, too (Phillippa, 2009).

South American countries have developed their own specialties. In Brazil, the cashew fruit juice is popular all across the country. In Panama, the cashew fruit is cooked with water and sugar for a prolonged time to make a sweet, brown, paste-like dessert. *Maranón* is one of the Spanish names for cashew.

Cashew nuts are produced in tropical countries because the tree is frost sensitive, adapting to various climatic regions between the latitudes of 25°N and 25°S (Arc, 2015). The traditional cashew tree is tall (up to 14 m) and takes three years from planting before it starts production, and eight years before economic harvests can begin. More recent breeds, such as the dwarf cashew trees, are up to 6 m tall, and start producing after the first year, with economic yields after three years. The cashew nut yields for the traditional tree are about 0.25 metric tons per hectare, in contrast to over a ton per hectare for the dwarf variety. Grafting and other modern tree management technologies are used to further improve and sustain cashew nut yields in commercial orchards. (*Cajucultura historia, 2010).*

In 2013, the world total for production of cashew nuts (in shells) was 4.4 million metric tons. Vietnam was the worldôs largest individual producer in 2013 with 1.1 million tons (FAO, 2013). As of 2014, rapid growth of cashew cultivation in Côte dôIvoire made this country the top African producer with nearly 500,000 tons (Bavier, 2014).

Fluctuations in world market prices, poor working conditions and low pay for local harvesting have caused discontent in the cashew nut industry (Embrapa, 2012).

Cashew oil is a dark-yellow oil for cooking or salad dressing pressed from cashew nuts (typically broken chunks created during processing). This may be produced from a single cold pressing.(*Indiana Public Media,* 2013).

2.1.11 CASHEW SHELL OIL

Cashew nutshell liquid (CNSL) or cashew shell oil (CAS registry number 8007-24-7) is a natural resin with a yellowish sheen found in the honeycomb structure of the cashew nutshell, and is a byproduct of processing cashew nuts. It is a raw material of multiple uses in developing drugs, antioxidants, fungicides, etc. It is used in tropical folk medicine and for anti-termite treatment of timber (Palermo, Elizabeth 2014). Its composition varies depending on how it is processed.

- Cold, solvent extracted CNSL is mostly composed of *anacardic* acids (70%), *cardol* (18%) and *cardanol* (5%) (Phillippa, 2009).
- Heating CNSL decarboxylates the anacardic acids, producing a technical grade of CNSL that is rich in cardanol. Distillation of this material gives distilled, technical CNSL containing 78% cardanol and 8% cardol; cardol has one more hydroxyl group than cardanol (Phillippa, 2009). This process also reduces the degree of thermal polymerization of the unsaturated alkyl-phenols present in cashew nutshell liquid.
- Anacardic acid is also used in the chemical industry for the production of cardanol, which is used for resins, coatings, and frictional materials (Glossary, 2014).

These substances are skin allergens, like the oils of poison ivy, and present danger during manual cashew processing (Palermo, 2014). This natural oil phenol has been found to have interesting chemical structural features which enable a range of chemical modifications to create a wide spectrum of bio-based monomers capitalizing on the chemically versatile construct, containing

three different functional groups: the aromatic ring, hydroxyl group and double bond in the alkyl chain. These can be split into key groups, used as polyols, which have recently seen a dramatic increase in demand for their bio-based origin and key chemical attributes such as high reactivity, range of functionalities, reduction in blowing agents and naturally occurring fire retardant properties in the field of ridged polyurethanes aided by their inherent phenolic structure and larger number of reactive units per unit mass (Palermo, 2014).

Cashew shell oil may be used as a resin for carbon composite products. CNSL-based Novolac is another versatile industrial monomer deriving from cardanol typically used as a reticulating agent for epoxy matrices in composite applications providing good thermal and mechanical properties to the final composite material.

Cashew apples in many parts of the world do not grow cashews, because the fruit, unlike the nut, is difficult to transport. Cashew apple juice, however, may be used for manufacturing blended juices.

2.1.12 ALCOHOL

In Goa, the cashew apple (the accessory fruit) is mashed and the juice extracted and kept for fermentation for a few days. Fermented juice then undergoes a double distillation process. The resulting beverage is called *feni or fenny*. Feni is about 40-42% alcohol. The single-distilled version is called *urrac*, which is about 15% alcohol.

In the southern region of Mtwara, Tanzania, the cashew apple (bibo in Swahili) is dried and saved. Later it is reconstituted with water and fermented, then distilled to make strong liquor often referred to by the generic name, gongo.

In Mozambique, cashew farmers commonly make strong liquor from the cashew apple, agua ardente (burning water).According to one source (Lamle, 2013).

Alcohol had been distilled in the early 20th century from the juice of the fruit, and was manufactured in the West Indies.

In the case of cashews, there is also some evidence showing cross-reactivity with peanuts, walnuts, and sesame seeds, such that persons suspecting food allergy to cashews may also want to determine the dietary safety and appropriateness of these other foods. These foods do not need to be eaten in their pure, isolated form in order to trigger an adverse reaction. For example, yogurt made from cowôs milk is also a common allergenic food, even though the cowôs milk has been processed and fermented in order to make the yogurt. Ice cream made from cowôs milk would be an equally good example.

Food allergy symptoms may sometimes be immediate and specific, and can include skin rash, hives, itching, and eczema; swelling of the lips, tongue, or throat; tingling in the mouth; wheezing or nasal congestion; trouble breathing; and dizziness or lightheadedness. Nut food allergy symptoms may also be much more general and delayed, and can include fatigue, depression, chronic headache, chronic bowel problems (such as diarrhea or constipation), and insomnia. Because most food allergy symptoms can be caused by a variety of other health problems, it is good practice to seek the help of a health care provider when evaluating the role of food allergies in your health.

2.2 THE FUNGUS, *ASPERGILLUS*

Aspergillus was first catalogued in 1729 by the Italian priest and biologist Pier Antonio Micheli. Viewing the fungi under a microscope, Micheli was reminded of the shape of an *Aspergillum*

(holy water sprinkler), from Latin Spargere (to sprinkle), and named the genus accordingly (Geiser, 2009). Today ñ*Aspergillus*ò is also the name of an asexual spore- forming structure common to all *Aspergilli*; around one-third of species are also known to have a sexual stage (Geiser, 2009). *Aspergillus* s*pecies* are molds, of which about 200 species have been identified. *Aspergillus* is known as a filamentous, cosmopolitan and ubiquitious fungi found in nature (Disalvo, 2000; Wood, 2002). About 16 species of *Aspergillus* molds are known to be dangerous to man (Madunagu and Umana, 2000).

The genus *Aspergillus* was originally divided into subgenera and groups (Raper and Fennel, 1965) but the current classification scheme replaces the designation ñgroupò with ñsectionò (Gams *et al.*, 1985) to conform to rules of the international Code of Botanical Nomenclaure. Currently, the genus *Aspergillus* is classified into 7 subgenera that are in turn sub-divided into several sections comprised of related species (Gams *et al.,* 1985). It was originally classified as Deuteromycotina (Fungi imperfect) but further research reassigned it to Ascomycotina (sac fungus) because its ability to form asci within closed ascocarps

(Porter, 2001). Clinical microbiology laboratories rely heavily on morphology-based identification methods for *Aspergillus* s*pecies* wherein diagnostic criteria include the recognition of asexual or sexual structures and their characteristics such as shape, size, colour, ornamentation and/or mode of attachment (Balajee *et al.,* 2007).

Aspergilli cause a wide spectrum of infections including cutaneous manifestations, otomycosis and invasive infections such as pulmonary aspergillosis and endocarditis. Pulmonary aspergillosis may range from invasive pulmonary aspergillosis (IPA) in severely immune-compromised patients to chronic necrotizing in mildly immune-compromised population (Pagano *et al*., 2007). *Aspergillus fumigatus* remains the predominant agent of IPA, followed by

wither *Aspergillus terraus* or *Aspergillus flavus* depending on the medical center. Recently, IPA due to *Aspergillus ustus* another rare aspergilla such as *Aspergillus* alliaceus (Balajee *et al*., 2007). *Aspergillus lentulus* (Balajee *et al*., 2005) and *Aspergillus udagawae* (Balajee *et al*., 2006) have been reported.

Aspergillus spores are common components of aerosols where they drift on air current, dispersing themselves both short and long distance depending on environmental conditions. When the spores come in contact with a solid or liquid surface, they are deposited and if conditions of moisture are right, they germinate, the ability to disperse globally in air currents and to grow almost anywhere when appropriate food and water are available means that ubiquitousô is among the most common adjectives used to describe these moulds (Bennett, 2010). Some *Aspergillus* species produce various mycotoxins. These mycotoxins, by chronic ingestion, have proven to possess carcinogenic potential particularly in animals. Among these mycotoxins, aflatoxin is well-known and may induce hepatocellular carcinoma (Mori *et al.,* 1998).

2.2.1 SCIENTIFIC APPLICATION OF *ASPERGILLUS*

The defining characteristic of the genus *Aspergillus* is the aspergillum-like spore-bearing structure. It is the most important microscopic character used in *Aspergillus* taxonomy. During mycelia differentiation, certain cells enlarge, develop a have cell wall and form ñTò or ñLò shaped ñfoot cellsô (which are not separate cells) that produce a single conidiophore perpendicular to the long axis of the cell. Sometimes it is difficult to see the foot ócellô but when visible, morphologists take it as strong evidence that an isolate in an *Aspergillus* species. The erect hyphal branch developing from the foot cell is the conidiophore, which enlarges at its apex to form a rounded, elliptical or club shaped vesicle. The fertile area of the vesicle gives rise to a

layer of cells called phialides (or steigmata in the older literature) that produce long chains of mitotic spores called conidia or conidiospores. The size and arrangement of the conidial heads as well as the colour of the spores they bear are important identifying characteristics (Bennett, 2010).

2.2.2 CLASSIFICATION

Kingdom: Fungi

Phylum: *Ascomycota*

Class: *Eurotiomycetes*

Order: *Eurotiales*

Family: *Trichocomacaeae*

Genus: *Aspergillus* (Geiser, 2009).

2.2.3 SOME ASPERGILLUS SPECIES

Some *Aspergillus* species are *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus tamari, Aspergillus nidulans. Aspergillus flavus* is the etiologic agent in a wide range of infections including mycotoxicoses owing to aflatoxins, hypersensitivity pneumonitis (Hoog *et al*., 2000), otitis (Jesenska *et al*., 1992; Harley *et al*., 1995), sinusitis (Drakos *et al*., 1993), and invasive disease. Some reports suggest the disease process may be potentiated by aflatoxins (Morie *et al*., 1996), particularly in the immune-compromised/ neutropenic host. Organism is extremely angioinvasive with resultant necrosis and infarction (Doctorfungus, 2007).

Aspergillus niger is the most abundant species of *Aspergillus* in nature as it can grow on a large variety of substances. *Aspergillus niger* is a cosmopolitan in soil and on plants. It commonly

causes ear infections (Fasunala *et al.,* 2008). It is also implicated in allergic aspergillosis, pulmonary aspergilloma and rarely primary cutaneous disease (Person *et al*,. 2010). Some strains of *Aspergillus niger* have been reported to produced to produce potent mycotoxins called ochratoxins (Abraca *et al*., 1994).

Aspergillus tamari is a member of *Aspergillus* section *Flavi* (Games *et al*., 1985). Although *Aspergillus tamari* is able to produce several toxic secondary metabolites, including cyclopiazonic acid and fumigaclavines (Samson *et al.,* 2004), It has rarely been encountered as a human pathogen. The only known cases are an eyelid infection (Degos *et al*., 1970), invasive nasosinusal aspergillosis in an immunocompetent patient (Paludetti *et al*., 1992), and onychomycosis in a three year old boy (Kristensen *et al*., 2005). To our knowledge, the present case of fungal keratitis is the first report on an ocular infection caused by *Aspergillus tamari* and the fourth known case worldwide involving this unusual opportunistic human pathogen (kredics *et al.,* 2007).

2.3 AFLATOXINS: HISTORY AND BACKGROUND

Aflatoxins are a special group of naturally occurring metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Sargeant *et al.,* 1961; Allcort *et al*., 1963). Over 40 species of *Aspergillus* have been listed as capable of producing toxic metabolites (Cole and Schweikert, 2003). Aflatoxins are toxic and among the most carcinogenic substances known (Machidia and Gomi, 2010). It has been classified as a class human carcinogen by the International Agency for Research on Cancer (IARC, 1993). Currently aflatoxins B1 is the major contaminant of food in tropical regions of Africa this been linked with hepatitis B and C infections and, to the high incidence of liver cancer in these regions (Montalto *et al.,* 2002). Growth of *A*spergillus species, spore and/or toxins production is affected by temperature, pH,

water activity, availability of air, and nutritional factors (Pitt and Hocking, 1985). Evidence for acute human aflatoxicosis has been reported from several underdeveloped countries such as India and Thailand. The symptoms of severe aflatoxicosis include oedema, hemorrhagic necrosis of the liver and profound lethargy (Williams *et al.,* 204).

Fungi-induced food toxicity has existed since early civilization. But mycotoxins and mycotoxicoses were relatively obscure and neglected in the scientific literature until the discovery of aflatoxins as the causative agent of Turkey-x disease in 1960 in England. The aflatoxin story began with the report of an outbreak of disease in turkey poults in England. Since the etiology of the disease was obscured it was called Turkey X disease (Blountm, 1961). Affected birds lost appetite, became lethargic and died within 7 days after the onset of symptoms. Lives of diseased turkeys were severely damaged. A similar disease of ducklings and young pheasants was reported from England (Aspllin and Carnaghan, 1961). A common factor in all disease outbreaks was the inclusion of Brazilian groundnut meal in the affected birdsô diets (Blount, 1961; Asplin and Carnaghan, 1961). A similar disease of duckling was reported from Kenya. The ducklings feed ration contained in ground nut meal reduced in eastern Africa, indicating that the problem was not solely associated with Brazilian groundnut meal (Allcroft and Carnaghan, 1962). Later in 1960 outbreak of disease occurred in pigs and calves, apparently caused from England (Asplin and Carnaghan, 1961). A common factor in all disease outbreaks was the inclusion of Brazilian groundnut meal in the affected birdsô diets (Blount, 1961; Asplin and Carnaghan, 1961). A similar disease of ducklings was reported from Kenya. The duckling feed ration contained a groundnut meal produced in Eastern Africa, indicating that the problem was not solely associated with Brazilian ground nut meal (Allcroft and Carnaghan, 1962). Later

in 1960, outbreak of disease occurred in pigs and calves, apparently caused by an unknown toxic factor in Brazilian groundnut meal contained in animal rations (Loosmore and Harding, 1961).

Sergeant *et al*., (1961) demonstrated that an isolate of the common mod *Aspergillus flavus* link ex fries was infact the responsible agent. The disease was caused by toxins produced by strains of the fungi *Aspergillus flavus* when growing on the meal, and hence these toxins, in view of their origin, were named aflatoxins. There were some reports of aflatoxicosis in farm animals from other European countries (Allcroft and Carnaghan, 1962).

Several outbreaks of aflatoxicoses in poultry have been reported from India (Char *et al.*, 1982). In 1962, a heavy mortality occurred among duckling in Tamil Nadu state (Bhat *et al.*, 1978). The feed used for the ducklings contained groundnut meal with a total aflatoxin contents of 6200µp $kg^{-1.}$ The aflatoxins (AF) are a group of difuranocoumarin compounds produced as secondary metabolites by the mold *Aspergillus flavus* and *Aspergillus parasiticus.* The major AF types found in plant food products are aflatoxin B_1 B_2 G_1, G_2 m_1 and M_2. Mycotoxin contamination in groundnut can occur in the field during pre-harvest, harvest and during postharvest handling (Nahdi, 1997). In many countries they are able to control the entry of contaminated groundnut in food chain by following strict regulatory programs. The maximum permissible limit of these mycotoxins varies from 0 - 100µg kg^{-1} depending on the country and food stuff and also whether the commodity is for human or animal consumption (Nahdi, 2000).

2.3.1 AFLATOXINS BIOSYNTHESIS

The biosynthesis pathway of the aflatoxins is quite well understood and has recently been described in a number of reviews (Bhatnager *et al.*, 1994; Trail *et al*., 1995; Bennett *et al*., 1997; Minto and Townsend, 1997; Kelter *et al*., 1997). Firstly acetate and malonyl CoA are converted

to a hexanoyl starter unit by a fatty acid synthase, which is subsequently extended by a polyketide synthase to the decaketide norsolorinic acid, the first stable precursor in aflatoxin biosynthesis. The polyketide then undergoes approximately 12 to 17 enzymatic transformations, through a series of pathway intermediates, including averantin 5^1-hydroxyaverantin, averufanin, averufin, 1ô hydroxyversiocolorone, versiconal acetate, versiconal and verisco lorin E. The pathway then branches to form AFB_1 and AFG_1 which contain dehydrobifuran rings and are produced from demethylsterigmatocystin (DMST) and in turn the other branch forms AFB_2 and AFG_2 containing tetrahydrobisfuran rings which are produced from dihydrodemethylsterigmatocystin (DHDMST). The proposed pathway is based mainly on evidence from enzymatic and genetic analysis.

Beside environmental factors, several metabolic promoters and inhibitor or aflatoxins biosynthesis has been reported (Gupta *et al.*, 1976). Similarly, some of the chelating agents that selectively bind with zinc or other stimulatory metals have been reported to inhibit aflatoxins production in *Aspergillus parasiticus and Aspergillus flavus*. These include phytic acid, dimethyl sulphoxide, 2-mercaptoethanol and tolnaftate, and may also inhibit the incorporation of acetate into aflatoxins. Many workers reported enhanced aflatoxins production in the presence of amino acids including thiamine, methionine, proline, tryptophan, asparagine, and alanine (Luchese and Harrigan, 1993).

The stimulatory effects of the various amino acids may be due to their effect on the growth and general primary metabolism of *Aspergillus parasitius* and *Aspergillus flavus* or even a direct effect on aflatoxin biosynthesis. The effects of antigungal agents from suppression of stimulation on levels of aflatoxins have aldo been reported (Moss and Frank, 1987).

Reports on the inhibition of aflatoxin production by benzoate and thioglycerol (Buchanan *et al*., 1986) and certain formulations of ammonium propronate (Magan and Lacey (1986) abound unsaturated but saturated longer chain fatty acid have also been found to stimulate aflatoxin biosynthesis (Tiwari *et al.*, 1986). The inhibitory effect of caffeine against aflatoxin biosynthesis while having no effect on growth of *Aspergillus prasiticsus* explain where this mould grows on ordinary and decaffeinated coffee beans but only produces aflatoxin on the later (Durakovic *et al*.,1985). Also the stimulatory and inhibitory effects of carbon sources and nitrogen sources on aflatoxin production have been widely discussed (Obidoa and Onyeneke, 1998; Ogundero, 1987; Luchese and Harrigan, 1993).

2.3.2 FACTOR PREDISPOSING *ASPERGILLUS FLAVUS* INFECTION AND AFLATOXIN CONTAMINATION

Some pre-conditions are essential for pre-harvest of *Aspergillus flavus* invasion and aflatoxin production. The foremost are:

(a) Presence of toxigenic strain of the fungus

(b) Susceptible host

(c) Favorable agro-climatic conditions.

Drought and temperature stress at critical stages in the life cycle of the crop is one of the dominant factors. Agronomic practices and insects play major role in *Aspergillus flavus* infection and aflatoxin contamination in field conditions (Diener, 1989).

2.3.3 UNDERSTANDING OF AFLATOXINS

Aflatoxins are a special group of naturally occurring metabolites produced by the fungi *Aspergillus flavus* and *Aspergullus parasiticus* (Sargeant *et al*., 1961; Allcroft *et al*., 1963). Over

40 species of *Aspergillus* have been listed as capable of producing toxic metabolties (Cole and Schweikert, 2003). Aflatoxins are toxic and among the most carcinogenic substances known (Machida and Gomi, 2010).

Production of aflatoxin on the agriculture commodities like groundnut, rice, wheat, corn, soybeans, cotton, and sorghum was done and found the rice was the best substrate (Hesseltine, 1965). It has been classified as a class I human careingoen by the international agency for Research on Cancer (IARC, 1993). Currently, aflatoxin B1is the major contaminant of food in tropical regions of African this has been linked with hepatitis B and C infectious and, to the high incidence of liver cancer in these regions (Montalto *et al*., 2002). Growth of *Aspergillus species* spore and/or toxins production is affected by temperature, pH, water activity (aw), availability of air, and nutritional factors (Pitt and Hocking, 1985). Evidence for acute humanaflatoxicosis has been reported from several underdeveloped countries such as India and Thailand. The symptoms of severe aflatoxicosis include oedema, hemorrhagic necrosis of the liver and profound lethargy (Williams *et al.*, 2004)

2.3.4 EXPOSURE TO AFLATOXIN

Human exposure of aflatoxin is difficult to avoid because *Aspergillus flavus* grows aggressively in many foods at all stages of the food chain in the field, in storage and homes (De Vries *et al.,* 2002). Acute exposure to high levels of aflatoxins can result in liver failure and rapid death. Chronic exposure, in both humans and animals, exacerbates infections disease and can lead to cancer, liver cirrhosis, weakened immune systems, and stunted growth in children (The International food policy Research Institute 2011). Previous work carried out by the consultative group on international Agriculture Research CGIAR) constituent internatila institute

of tropical Agriculture (IITA) has shown that 99% of children at weaning age in Benin and Togo are highly exposed to serious health risk linked to aflatoxin leading to reduced growth and immunue- responses. Other common symptoms of acute poisoning by AFB include depression and anorexia, as seen in the recent contamination of pet food in south Texas (Garland and Reagor, 2001) and South Carolina (Lang, 2006). A study by (Turner *et al*., 2003) in Gambian children showed evidence that secretory IgA in saliva may be reduced to dietary aflatoxin exposure importantly, (Gong *et al.*, 2002, 2004) and Tuner *et al*., (2007) reported an association between biomarkers of aflatoxin exposure and growth impairment in children in West Africa. After ingestion, aflatoxin is metabolized by cytochrome p450 group of enzymes in the liver, where it is converted to many metabolic products like aflatoxicol, aflatoxin Q1 aflatoxin P1, and aflatoxin MI, depending on the genetic predisposition of the species. Along with the above another metabolite decides the species susceptibility as this can induces mutations by interrelating in to DNA by forming and adduct with guanine moiety in the DNA (Smela *et al*., 2001). This interrelation of expoxide causes a Ga T transversion at codon 249 inp.53 gene in liver, which may lead to hepatic caracinoma. This was observed in most of the experimental model and it is presumed that this is the major reason for aflatoxin carcinogenecity (Katherine *et al*., 1997; Railey *et al.,* 1997). According to the United Nation Food and Agriculture Organization (FAO), 25% of world food crops are affected 5 billion people are potentially exposed to aflatoxin in the developing world.

2.3.5 HEALTH IMPLICATIONS OF CONSUMING CASHEW NUTS CONTAMINATED WITH AFLATOXIN

The consumption of mycotoxin-contaminated cashew nuts is related to several acute and chronic diseases in humans as well as in animals. While the exact cause and effect relationship has been

established for only a few of the diseases, speculation about the role of mycotoxins in the aetiology of various illnesses have been identified. There is some evidence of an association with mycotoxins include: aflatoxic hepatitis in India and Kenya, enteric ergotism in India; vascular ergotism in Ethiopia, and deoxynivalenol mycotoxicosis in India and china. A common feature in all these outbreaks has been the involvement of staple foods such as corn, wheat or pearl millet, following unseasonable rains or drought during either the growing season or harvest (Bhat and Miller, 2014). Among the mycotoxin, aflatoxins have been implicated in human disease including liver cancer, Reyeôs syndrome, Indian childhood cirrhosis chronic gastritis, kwashiorkor and certain occupational respiratory disease in various parts of the world, Particularly in African and Asian countries. In China the Philippines, Thailand, Kenya., Swaziland and Mozambique, higher levels of aflatoxin in the food supply have been correlated with aflatoxin and their derivatives in human fluids which may be associate with liver cancer (Palmgren and Hayes, 1987), *Fusarium* toxins have been suspected to have a role in disease such as Kashin Beck syndrome in the USSR, Chain and Viet Nam; mseleni joint disease in southern Africa; endemic familial arthritis in India; alimentary toxic leukamia in the USSR, and oesophageal cancer in Southern Africa. Ochratoxins have been associated with Balkan endemic nephropathy and urinary tract tumours (Berry, 1988).

2.3.6 TOXICOLOGY OF AFLATOXINS

Broiler chicks show depressed weight gains and liver lesion after 10 weeks on a diet containing 200ppb AFB_1 but laying hens are said to be unaffected after 33 weeks on a diet containing 610 ppb. For weaning swine, the no effect chronic level was found to be 40 ppb in the diet and concentrations greater than this had demonstrable effects. Beef cattle are said to show no effects at concentrations in diet below 44ppb but liver damage is demonstrable at 700ppb. The Shasta

stain of rainbow trout shows liver tumors when exposed to a diet containing as little as 0.5ppb after 20 months, whereas the Coho salmon is apparently unaffected by as much as 200ppb. Differences in toxicity of AFB_1 to the male and female of a single species and difference between species arise because the mycotoxin is metabolized by the liver and expression of toxicity according to Moss (1996), depends on the dynamics of this process

The physio-chemical properties of aflatoxins B_1 are studies for the effective delivery of the toxin from the diet to the cytoplasm of the liver cells. Once there, it may be converted to the epoxide, a good candidate for the carcinogen and known to react with guanyl residue in DNA. The epoxide, itself may be further metabolized by an epoxide hydratase to form a dihydroxy compound which is a good candidate for the acute toxin and known to react with proteins. The implications of aflatoxins metabolism for the expression of disease have been reviewed by Neal (1987).

A diverse range of responses to the toxic effects of a compound may occur because the compound is metabolize in the animals body and the resulting toxicity is influenced by this metabolic are activity. This is certainly the case with AFB_1 form which a very wide range of metabolites are formed in the livers of different animal species. Thus the cow is able to hydroxylate the molecule and secret the resulting aflatoxin M_1 in the milk, hence affording a route for the contamination of milk and milk products in human foods even though these products have not been moulded (Neal, 1987; Egmond, 1989; Aksit *et al*., 1997).

The formation of an epoxide could well be the key to both acute and chronic toxicity and those animals which fail to produce it are relatively resistant to both. Those animals which produce the epoxide, but do not effectively metabolize it further, may be at the highest risk to the carcinogenic activity of AFB_1 because the epoxide is known to react with DNA. These animals

which not only produced epoxide but effectively remove it with a hydrolase enzyme, thus producing a very reactive hydroxyl acetate, which are most sensitive to the acute toxicity. The hydroxyl acetate is known to react with protein.

The parent molecule may thus be seen as a very effective delivery system having the right properties for absorption form the gut and transmission to the liver and other organs of the body. It is however, the manner in which the parent molecule is subsequently metabolized in vivo which determines the precise nature of an animal response (Moss 1996).

2.3.7 AFLATOXINS AND PRIMARY LIVER CANCER (PLC) IN MAN

Aflatoxins are one of the most carcinogenic compounds known for rat and rainbow trout (Moss 1989; Ramos and Fernandez 1996). Liver cancer is such parts of the world as the African continent is complex and it is probable that aflatoxin is only one factor in the appearance of the disease. There is a good correlation between the presence of hepatitis B virus and PLC in man and there is an increasing consensus that these two agents act synergistically to elicit hepatocarcinoma (Hsieh 1986; Olubuyide *et al.*, 1993; Blum 1994; Chao *et al*., 1994; Diallo *et al*., 1995; Bhat 1996; Yu *et al*., 1997; Dhir and Mohandas 1998).

2.3.8 OTHER TOXIC EFFECTS DUE TO AFLATOXIN

a. Humans:

Aflatoxins have been shown to be risk factors either experimentally or by circumstantial evidence in many disease entities in man. For example, several studies have shown significant levels of aflatoxins and aflatoxicols in the blood and other body fluids of children in the tropics and it has been proposed that aflatoxin in the diet of children is associated with kwashiorkor (Hendrickse, 1984; Coulter *et al.,* 1986). Very young children can be exposed to aflatoxins even

before they are weaned because mothers consuming AFB_1in their diet can secrete AFM_1 and AFM_2 in the breast milk (Coulter *et al.*, 1986; Moss 1996). Coulter *et al*. (1984), detected both toxins in the breast milk of Sudanese women, and also demonstrated same in the breast milk samples of mothers attending maternity hospital in Abu Dhabi.

Aflatoxins have been shown to exhibit several other toxic effects such as inhabitation of protein synthesis, inhibition of mitochondrial energy metabolism, and the induction of blood clotting disorders. Thus, according to Hendrickse (1991), the inhibition of protein synthesis is believed to account for the reported role of aflatoxins in the genesis of kwashiorkor. Aflatoxins have also been reported as a cause of acute hepatitis in a number of cases from developing countries (Krogh, 1987). In Zaria and Ekpoma, Nigeria, in two prospective studies, a possible relationship was shown between perinatal aflatoxin exposure and neonatal jaundice, though no relationship was shown between the severity of hyperbilirubinaemia and serum aflatoxin levels (Ahmed *et al*., 1995; Abulu *et al*., 1998). In Ibadan, Nigeria, Sodeinde *et al*. (1995) observed that the presence of any serum aflatoxin was a risk factor for neonatal jaundice.

Jonsyn *et al*., (1995) in Serria Leone observed that the presence of aflatoxins in maternal blood samples contributed to the low birth weight of their infants. De-Vries *et al*., (1989) noted that the mean birth weight of infants born of aflatoxin-positive mothers in Kenya was significantly lower (255kg) then those born to aflatoxin free mothers. Two still births were also recorded and these results coincided with the adverse-effects of prenatal aflatoxin exposure recorded in animal experiments.

Dietary exposure to aflatoxins in Benin City, Nigeria has been associated with human male infertility. Infertile men with aflatoxins in their semen showed a higher percentage of spermatozoa abnormality (50.0%) than fertile men (10.0-15%) (Ibe *et al.,*1994). Aflatoxins have

also been revealed to act as protein genotoxins causing some forms of genetic alternations, including chromosomal aberrations, micronuclei and sister chromosomal aberrations and other forms of genetic damage (Harrison *et al*., 1993; Miele *et al*., 1996). Hollstein *et al.* (1997) also noted gene P53 alterations in human tumours induced by aflatoxins.

Increase in prothrombin time, decreased in total plasma proteins and albumin level and increase in red blood cell counts and haematocrit have been demonstrated by Fernandez *et al.* (1995) .Robens and Richard (1992) and also acute hepatic failure and Reyeôs syndrome due to aflatoxins in their patients. Further researches have also shown that aflatoxins increase childhood susceptibility in infections, compromise immune responses to prophylactic immunizations and also may generally have the potential of immunosuppression and immunotoxicity (Hendriskse, 1991; Neal *et al*., 1998; Gabal and Dimitri, 1998). Aflatoxin involvement in digestive tract cancer and lung lesion has also been reported (Oyelami *et al*., 1997).

b. Animals

In all species of domestic animals, aflatoxicoses has been well documented in which the evidence of disease is a general unthriftness and reduction in weight gain, feed efficiency, immunity and production (Robens and Richard 1992; Moss 1996). More conclusive evidence in aflatoxin involvement in animal disease include acute to chronic liver disease with concomitant increase in specific liver enzymes in the serum. In cattle, milk production is affected, but of greater significance is that the aflatoxins in feeds can be rather efficiently converted to toxic metabolite readily detectable even in small amounts in milk. The poultry industry probably suffers greater economic loss than any other livestock industry because of the greater susceptibility of their species to aflatoxins than other species (Robens and Richard, 1992).

Fernandez *et al*. (1996) observed significant reduction in body weight and in the average daily weight gain in the intoxicated group of lambs when compared with a control group. In his experiments, lambs fed with aflatoxins had relatively smaller liver weights and higher kidney and spleen weights than lambs from the control group. Blood parameter, urea levels, alkaline phosphatase and glutamate dehydrogenase enzymatic activities were also affected as a result of the intoxication. Change in the coagulation profile of the lamb intoxicated with aflatoxins was also observed. Similar observations were also noted for hens and broiler chickens including changes in prothrombin time, total plasma and albumin levels, haematological values and red cell counts (Fernandez *et al.*, 1995).

In further studies in lambs, Fernandez *et al.* (1997) observed that aflatoxins cause a failure in the acquired immunity system of lamb by decreasing antibody production and altering serum profile proteins.

2.3.9 EFFECT ON TRADE

The application of different permissible levels in different countries (e.g. European Union countries, the USA, and developing countries) has tremendous impact on international trade in various agricultural commodities such as groundnut, maize. Cashew nuts, pistachios, copra and chilies. The harmful effects of mycotoxins on animalsô productivity and on human and animals health justify stricter control of mycotoxin levels in foods and feeds (Mehan and Jand, 2002).

The significance of mycotoxins in international trade is increasingly being recognized by both developed and developing countries. The export of agricultural commodities such as copra, cottonseed, peanuts and pistachio nuts or their derivatives have been affected. Often and especially in developing countries, the best quality of these commodities, which are free from

mycotoxins, are exported, while the substandard products are distributed and sold within the country. This practice has the potential of unfavourable consequences in either the health of the local population or productivity of the animals fed with contaminated or substandard feed (Dawson, 1991). Thai maize has been noted for its bright yellow colour and high protein content. However, sample has been found to contain unacceptably high aflatoxin levels and are therefore discounted or rejected by foreign buyers (Cutler, 1991). Hence aflatoxin contamination has posed serious problems in commerce and international trade because of stringent quality standards on aflatoxin contamination by importing countries (Bhat, 1988). Mycotoxin concentration may exceed government standards for trade and beyond unit for human consumption.

2.4 ENVIRONMENTAL AND BIOLOGICAL FACTORS INFLUENCING FUNGAL ATTACK AND THE PRODUCTION OF AFLATOXINS

An important aspect of the control of mycotoxins is to identify the factors that contribute to contamination of crops. These factors are outlined below broadly.

- **Nature of the Crop Substrate**

The substrate is a determining factor in the development of most fungi. Some commodities are more susceptible to contamination by certain moulds than others while most moulds can however, contaminate many substrates. The nuts and grains are considered to be an ideal substrate for the growth of fungi providing the necessary carbohydrate for energy and fats for additional energy (FAO, 1997) of all the agricultural crops studies in Nigeria, the most susceptible of aflatoxin combination are groundnuts (Crowther, 1973; Adebajo, 1994) maize (Nwokolo and Okonkwo, 1978; Opadokun 1990; Adebajo *et al*. 1994) and copra (Obidoa and

Gugnani, 1990). Cashew nut appears to be one of the most susceptaible crops to contamination by various fungi and mycotoxins internationally (Lovelance and Aalbersberg, 1989; Wood 1989; Price *et al*., 1993; Arim, 1995). Even with the same crop, certain cultivars appear to be more resistant to fungal attack then others. These include groundnuts (McDonald, 1976), cowpeas and maize (El-Kady *et al*., 1996).

- **Condition of the Cashew Nuts**

Mature, whole unbroken nuts are less susceptible to mould attack than immature, broken and defective nuts (Haq-Elamin *et al*., 1988). Adebajo *et al*., (1994) observed that the incidence of mould contamination was generally higher in shelled water melon seeds than for unshelled seed samples. Doster and Michailides (1994) got more than three times higher aflatoxin load and *Aspergillus flavus* contamination in the early splits of Pistochio units than from normal units with intact bulls. The early splits are atypical nuts that have split hulls exposing the kernel to invasion by moulds.

- **Strain of Fungus**

Fungus may attack food crops without necessarily producing any mycotoxin. This is because not all stains of fungus that attack crops produced toxin. Moss (1996) noted that on a worldwide basis, only about 35% of stains of *Aspergillus flavus* produce aflatoxins while FAO (1997) earlier noted that only 58% of all stains produced aflatoxin. Many other workers have also observed the same trend Mishra and Daradihiya (1991); Lacey (1989) in his studies noted that only 42,3% of all his *A*spergillus *flavus* isolates produced aflatoxins. It has also been shown that isolate of *Aspergillus parasiticus* produce aflatoxins in higher concentrations than *Aspergillus*

flavus which contains a greater percentage of non- aflatoxigenic strains and produced only B aflatoxins (Pitt, 1993).

- **Temperature**

Fungi can only attack crops successfully within certain temperature limits (between O^0 and 60^0C) in general, low temperatures reduces the rate of fungal growth. All fungi have characteristics minimum, optimum and maximum temperature for growth. Certain fungi like *Fusarium species and Cladosporium* species grow and produce toxins at rather low temperature (10^0C and 20^0C), while *Aspergillus flavus* grows at temperature between 12^0c and 42^0C but aflatoxin production is maximum at temperature between 25^0C and 32^0C (Koehler *et al*., 1985; Pitt and Hocking, 1997). These temperatures approximate the ambient temperature in most part of Nigeria most of the year. Most fungi growing on plants before harvest (the field environment) grow well in the temperature range 0 to 30^0C (Lacey, 1989), while most fungi found in the storage environment, however, thrive within the range 10 ï 40^0C (Optima 25 ï 35^0C*).* *Aspergillus fumigatus* has an exceptionally wide range from 10 - 55^0C while some Ascomycetes have ascospores which are very resistant and able to survive ten minutes heating at 90^0C.

- **Moisture**

Based largely on moisture requirement for growth, fungi that attack crops can be grouped into field fungi that attack grains at moisture level exceeding 30% (e.g. *Alternaria, Fusarum* and *Cladosporum* species*),* and storage fungi that prediomiante in grains in the 13% to 18% moisture range (e.g. *Aspergillus* and *Penicillum* groups) the moisture content of the grains which is in equilibrium with air is referred to as ósafeô moisture content for which Agboola (1990), has enumerated for the storage of some Nigerian food crops at 20^0C follows:

Crop	Safe moisture contents at 27^0C
Cowpea	15.0
Maize	13.0
Millet	16.0
Rice	13.0
Sorghum	13.0
Ground nut (shelled)	7.0
Ground nut (unshelled)	9.0
Soybeans	8.0
Garri	12.0
Cashew nuts	5.4

Lacey (1989) has also shown that even when dry grains are put into store, there is possibility of exchanging of moisture in the vapour phase due to temperature gradients produced by fluctuating external temperature. This can contributed to even a higher fungal growth during storage.

2.4.1 OCCURRENCE OF AFLATOXINS AND FUNGI IN OIL SEEDS

In all parts of the world, reports abound on the distribution of moulds and their consequent production of aflatoxins in oil seeds. Johnsyn (1988) isolated toxigenic species of *Aspergillus flavus, Aspergillus ochraceus, Aspergillus tamari, Penicillium citrinum* and *Fusarium* species from sesame seeds in Sierra Leone and AFB_1 and AFG_1 and other fungal toxins were positively identified. In Sudan, Hag Elamin *et al*., (1988) observed that levels of aflatoxins were distributed according to geographical climatic feature. They also showed that from the samples

analysed, that damaged pods had more levels of aflatoxins and mould contamination when compared with intact sound pods. According to them also, gray and red roasted pods also showed higher amounts of aflatoxins while groundnut paste was the least contaminated.

Groundnut samples from 21 selected markets in the 10 regions of Ghana yielded high levels of aflatoxigenic. *Aspergillus flavus* was associated with 31.7% and 12.8% respectively of all damaged and undamaged kernels assayed. Other fungi detected included *Aspergillus niger 34%, Aspergillus candidus 1.45%, Aspergillus tamari 3.9%, Aspergillus ochraceus 5.26%, Fusarium* species *1.7%, Penicillim* species 5.19%, *Mucor* species *2.3%, Trachoderma spp* 0.2%, *Rhizopus stolonifer* 12% and certain unidentifiable fungi (11.72%). Aflatoxin levels of 168ppb were identified with damage samples while none was detected in 50% of undamaged samples tested and very low levels of aflatoxins ranging from 0.1 to 12.2 ppb were associated with these undamaged samples (Awuah and Kpodo, 1996).

Amongst the oilseeds, groundnuts are easily the most susceptible to aflatoxin contaminations in all samples analysed (Crowther 1973). In Nigeria studies show that over 60% of the crops examined showed aflatoxin levels higher than the allowable limited by the FAO (Food and Agricultural Organization 1997) i.e, 30ppb. According to Obidoa (1975), the food crops most commonly infested by aflatoxigenic fungi include groundnuts, cereals, cocoa, coconuts, fruits and root crops with aflatoxins being detected with highest levels in the oil seeds and nuts such as groundnut and copra. Obidoa and Obasi (1991) reported the aflatoxin levels of table foods sold in public eating places at Nsukka including garri, egusi, ora and bitterleaf soup and other foods as ranging between 99.84 to 268ppb for

A total of 4532 samples of sovietunion domestic and imported cereals and other foods were sampled for aflatoxin contamination. The results showed that 26.9% of peanuts, 2.2% of corn

and 28.3% of cotton seeds were contaminated with aflatoxins at levels exceeding the maximum tolerated levels established in the USSR, (5mg/kg) for AFB_1 in all food stuff (Tutelyan *et al*., 1989). Munir *et al.* (1989) in Pakistan observed that only 41.6% of maize samples and 2.5% of red chilies assessed were contaminated with levels of 11.12 ï 82. 23mg/kg for AFB_1 and 41.6mg/kg for AFB_2. In the United States of America, food commodities including peanuts, peanuts products, tree nuts, cotton seed, milk, spices and other miscellaneous foods were analysed by Wood (1989). Correlations were highest between aflatoxin contamination and geographical areas for corn and corn product and cotton seed/cotton seed meals

Sohri and Saber (1993) isolated 59 species and one variety belonging to twenty, five genera of fungi in Egypt form twenty nine samples of coconut. The fungi include *Aspergillus flavus, Aspergillus niger, P. chrysogenum, C. cladoporoideas, Alternaria alternate, Rhizopus stolonifer, Trichoderma hamatum* and *Eurotiumk chevaleri.* Analysis showed that five samples tested of the twenty nine were naturally contaminated with AFB_1 (15- 25mg/kg) and three sample were contaminated with Ochratoxin A (50 ï 205mg/kg). Price *et al*. (1993) detected that 10 out of 644 (1.6%) domestic corn samples and 7 to 106 (6.6%) domestic cotton seed samples contaminated aflatoxins at levels greater than 300 ppb in a survey of naturally occurring toxins in feedstuff in Rockville. Russell *et al*. (1991) also noted that *Fusarium* species were the most predominant in maize samples. In the In the Philippines, a survey on the aflatoxin contamination of various foods indicated that corn and peanuts were the two commodities that contained toxic levels of aflatoxins (Arim, 1995).

A study was carried out to investigate the microbial and aflatoxin contamination of cereals and cereals products in Bangkok, Thailand, result showed that rice and rice products were free form bacteria, one sample was contaminated with AFB_1 (10ppb) and four with AFG_1 (20 ï 50ppb).

Two of the four samples cultured for fungus revealed *Aspergillus niger*. Out of the 30 samples of peanut products, bacterial contamination included only bacillus species and 13(43%) contained aflatoxins (40 ï 780ppb) with a mean of 130 ppb and AFG_1 (130 ï 160ppb). *Aspergillus flavus* was found in 8 samples and *Aspergillus niger* in 2 of the 13 samples. The soybeans samples 10 soybean oil examined were age, 4 of 10 samples of fermented soybeans were contaminated with AFB_1 level varying from 10 ï 20 ppb and AFG_1 20 ï 100 ppb and 2 of the 4 sample revealed *Aspergillus niger*. Beans samples which include garden pea (lantoa bean), fava bean, pigeon pea, red bean, black bean and green beam were studied. One sample of lantao bean contained 50 ppb AFT_1.

2.5 AFLATOXICOSIS IN ANIMAL AND HUMAN

Mycotoxin is derived from the Greek word ñmykesò meaning fungus and the Latin world ñtoxicumò meaning poison (Forgaes and Carll, 1962). Mycotoxins are secondary fungal metabolites that cause pathological or undesirable physiological responses in human and other animals. Mycotoxicoses are diseases caused by the ingestion of foods or feed contaminated by mycotoxins (Goto, 1990). Aflatoxins have received greater attention than other mycotoxins because of their established carcinogenic effect in various animals and their acute toxicological effects in human (Mehan, 2002).

The term aflatoxin is derived from *Aspergillus flavus.* A for *Aspergillus, flavus* for *flavus*, and toxin as a descriptor of the substance. Mycotoxins result in both acute and chronic toxicities. Acute effects result in rapid, readily noticeable fatal disease. Some mycotoxins are acutely toxic to the liver. While other attack the kidney, central nervous system or circulatory sytem. For example aflatoxins B_1 is a potent liver toxin and less than 20µg has a lethal effect on ducklings.

The chronic effect results from the carcinogenicity of aflatoxins B_1 diet containing only 0.1 $\mu g/kg^{-1}$.Aflatoxins B_1 resulted in liver tumors in rainbow trout (Coker, 1984).

Mycotoxins produce toxic reactions in animals by contact or inhalation. Intake of low mycotoxin concentration reduce mental alertness, physical ability and feed intake. Intake of moderate concentration reduced the activity of the immune system and increased susceptibility to other disease (Agarwal and Sinclair, 1977).

Physiological damage by mycotoxin varies with individual species, their age, state of health, degree of exposure and other factors (Agarwal and Sinclair, 1977).

Occurrence of mycotoxicoses is governed by the quantity of toxic food ingested time lag in symptom development, toxic concentration in the food, sensitivity and age of the individual, sex, nutritional status, season of harvesting, weather and altitude of the crop production area (Jofe, 1978).

Mycotoxicoses has four clinical symptoms:

(a) It is non communicable

(b) Drugs and antibiotics are ineffective

(c) Outbreaks are associated with a specific food or feed

(d) Foodstuffs show active fungal growth upon examination (Detroy *et al*., 1971).

Animal health: Aflatoxicosis caused by the presence of aflatoxins in feeds is a serious syndrome in poultry. Several species of molds are involved in spoilage of both raw and processed poultry feeds. The commonly occurring storage molds-

Aspergillus parasiticus and *Aspergillus flavus* can invade maize, groundnut and fish meal-based feeds and consequently produced highly toxic and cancer-causing aflatoxins (Narsapur, 2002).

The adverse effects of different mycotoxins vary widely due to their diverse chemical structure. High concentrations of mycotoxins produced immediate overt mycotoxicoses while low concentrations of mycotoxins are predominantly associated with immunosuppressive activity in domestic livestock and poultry flocks where active immunization schedules are an important part of the animal health programme. Aflatoxins consumption has been found associated with increased susceptibility to coccidiosis and faciolosis in domestic animals. Immuno-supression due to mycotoxins often causes vaccine failures in livestock and poultry (Juyal, 2002).

Aflatoxins are capable of producing liver cancer in the most sensitive animal species when fed at a concentration of only one part per billion (ppb). Ducklings, chicks, calves, guinea pigs and pig trout are very sensitive to the hepatoxoine effects of aflatoxins B_1 whereas rat, goat sheep and mouse are relatively less sensitive to the acute effects of this toxin (Mor and Singh, 1998).

Animals which consume sub-lethal quantities of aflatoxins for several days or weeks develop a sub-acute toxicity syndrome which commonly includes moderate to severe liver damage. Lethal dietary aflatoxins levels in domestic animals range from 0.3 mg kg^{-1} in the ducklings to 2.2 kg^{-1} in valves (Allcroft, 1965). Similar result has been obtained in laboratory animals. Including the guinea-pig and rat, which develop toxicity symptoms at 0.7 and 3.4 mg kg^{-1} respectively (Butler, 1964).

Early signs of aflatoxicosis in livestock are reduction in feed intake, and weight loss, this is often rapidly followed by death. The most important pathological effect is liver damage.

Human Health: One of the most impressive aflatoxin-related episodes reported in the scientific literature is an acute poisoning in an area in India in 1974 involving some 400 people and

resulting in 106 deaths (Van, 1977). The circumstances were typical of those highly conductive to excessive mycotoxin exposure. They consumed aflatoxin contaminated corn kernels.

Epidemiological studies have been carried out to see if aflatoxins ingestion might be a factor in the high incidence of liver cancer in some area of Africa, Asia and the USA. Several studies in Indonesia, Kenya, Mozambique, South Africa, Swaziland, Thailand and Uganda found a positive correlation between aflatoxin ingestion by humans and liver cancer incidence (Husaini *et al*., 1974; Peers *et al*., 1976; Van *et al*., 1974; Keen and Martin, 1971; Shank *et al*., 1972).

Some researchers have highlighted the strong relationship between hepatitis B virus infection and incidence of liver cancer (Beasley et al., 1981; Beasley 1982). All epidemiological studies of aflatoxins and liver cancer conducted in Africa and Asia involved population subjected to hepatitis B virus infection. Workers engaged in harvesting, shelling, bagging storage, marketing, and transport of groundnuts may be exposed to aflatoxin through the respiratory route. A chemical engineer involving in sterilizing Brazilian groundnut meal contaminated by *Aspergillus flavus* developed alveolar cell carcinoma and died within a year. Two reports of aflatoxicosis in humans involve consumption of maize heavily contaminated with aflatoxins (Krishnamachari *et al*., 1975; Nagindu *et al*., 1982). In some of the incidents in India there were 272 hospital admission with clinical symptoms of aflatoxicosis and 27% mortality. In the second incident, in Kenya, there were 20 hospital admissions with a 60% death. The presence of aflatoxin in dairy milk is of great public health significance because they are potent toxins, carcinogens and mutagens. These toxins cause serious health hazards to human including acute toxicity, liver cancer and immune-suppression. Infants and children are highly susceptible to the toxic effects of aflatoxins. Dairy milks is a major component of the diet of children and there are reports that

children who consumed aflatoxin contaminated milk developed Indian childhood cirrhosis (an inflammation of liver). It should also be noted that women consuming aflatoxin contaminated food can have aflatoxin in their breast milk, a direct threat to the health of the infants. The amount of aflatoxin M_1 excreted in milk is in direct proportion to the intake of aflatoxin B_1 quantitatively about 1.5 percent of the aflatoxins B_1 ingested by a cow appears in the milk as m_1 (Jand and Dhand, 2002).

Amal *et al*. (1970, 1971) presented circumstance evidence to indicate that children exposed to aflatoxin through breast milk and dietary items such as unrefined groundnut oil and parboiled rice may develop cirrhosis. They detected aflatoxin B_1 in 7% of urine sample from cirrhotic children.

The table below shows the current European Union legislative limits (EU Commission Regulations 446/2001, 257/2002 and 477/2002

Product type	Aflatoxins: maximum admissible levels (μg/kg)	
	B_1	Total aflatoxins (B_1 + B_2 + G_1 + G_2)
Groundnuts, nuts, dried fruit and processed product for direct human consumption or as a god ingredient	2	4
Groundnut to be subjected to sorting, or other physical treatment, before human consumption or as a food ingredient	8	15
Cashew uts and dried fruit to be subjected to sorting or other physical treatment before human consumption or as a food ingredient	5	10
Cereals and processed products thereof for direct human consumption as a food ingredient	2	4
Chilies chili powder, cayenne pepper, paprika while & black paper nutmeg, ginger turmeric	5	10

2.6 PREVENTION AND CONTROL

The presence and growth of *Aspergillus* species on pre-harvested crops is dependent on the environment. Agricultural practice including proper irrigation and pest management can reduce aflatoxin contamination. Pre-harvest interventions include choosing crops with resistance to drought, disease and pests and choosing stains of that crop which are genetically move resistant to the growth of the fungus and the production of aflatoxins (Chen *et al*,. 2001). Elimination of innoculum source such as infected debris from the previous harvest may prevent infection of the crop (Olanya *et al*., 1997). A bio-pesticides, consisting of a non-aflatoxigenic strain of *Aspergillus*, may competitively exclude toxin strains from infecting the crop (Dorner *et al*., 1999; Cleveland *et al*., 2003) however, the allergenic and human health aspects of the aflatoxigenic strain need to the assessed

Before storage, crops should be properly dried to prevent the development of aflatoxins. Sorting and disposing of visible moldy or damaged kernels before storage has proven to be an effective method for reducing by not eliminating the development of aflatoxins (Fandohan *et al*., 2005; Turner *et al*., 2005). During storage, moisture, insect and rodent control can prevent damage to the crop and reduce aflatoxin development. A community-based interventions trail in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in man aflatoxin levels in intervention villages (Turner *et al*., 2005).

In order to prevent future outbreaks, developing countries need an early warning system which is able to detect potential food contamination events with adverse health effects (Park, 1995). Monitoring aflatoxin levels in good individual to identify those at risk for disease is more difficult than monitoring rates of jaundice. However, food and biological monitoring may

identify susceptibility sooner and allow for a more timely intervention, to maximize resources, monitoring or surveillance should target high-risk areas or populations and the most appropriate specimen-food urine, or serum-should be collected. A rapid field test that analyzes aflatoxin in biological samples would be ideal for early warning system that incorporates bio-monitoring. Aflatoxin metabolites in urine reflect recent exposure (i.e 2-3 days) whereas over a longer period (i.e 2-3 months) (Groopman *et al*., 1994). These analysis, however, are labor-intensive and expensive (Sheabar *et al*., 1993).

CHAPTER THREE

MATERIALS AND METHODS

This study adopted the survey and the experimental design.

3.1 STUDY AREA

This study was carried out in Enugu, the capital of Enugu State Nigeria. Enugu has a typical climate with two distinct seasons: the dry season (often lasts from November to march) and rainy season (April to October).Roasted cashew nuts are among the most traded snacks throughout the season in Enugu and the business is favoured by high population and commercial activities of the town. However, the high temperature and humidity of the area are important climatic conditions for the incidence of aflatoxin contaminations of poorly processed cashew nuts which constitute high proportion of food vendorôs commodities in the city.

3.2 SAMPLE COLLECTION

A total of 152 samples of roasted cashew nuts were obtained from different locations in Enugu metropolis. The physical conditions of the packaging materials and of the nuts were carefully noted. The cashew nuts were categorized into two groups viz; exposed and unexposed

3.3 SAMPLING GROUPS

Groups	**Number sampled**
Exposed roasted cashew nuts	62
Unexposed roasted cashew nuts	90

The exposed cashew nuts were roasted cashew nuts hawked without being packaged in hand-knotted thin polyethylene bags in wheel barrows by market women. **The unexposed** cashew nuts were roasted cashew nuts packaged in hand-knotted thin polyethylene bags.

3.4 EVALUATION OF VISIBLE MOULD INCIDENCE

The total of 152 samples of cashew nuts were carefully observed with the naked eyes for any evidence of mould growth. The cotyledons (2 per nut) were then separated by hand and the state of the inner surface was also noted. The incidence of visible mould contamination was expressed as a percentage of 152 nuts examined.

3.5 SAMPLING METHOD

Two cotelydons from each sample were randomly picked and surface sterilized with a 2% aqueous solution of sodium hypochlorite for 2 minutes. This was followed by rinsing with three washes of sterile distilled water before the two cotyledons were plated together equi-spaced from each other on Sabouraud dextrose agar containing chloramphenicol (50mg/ml) plates.The plates were incubated at 28^0c for 5 ï 8 days after which the number of cotyledons that yielded colonies were recorded, enumerated and sub-cultured for identification of *Aspergillus flavus.* Fungal incidence was expressed as a percentage of the 152 cotylendons plated.

This method of fungal culture was in accordance with Pitt and Hocking (1985). The petri dishes were then carefully examined after 5 ï 8 days for colonial growth.

3.6 IDENTIFICATION OF FUNGAL ISOLATES

Identification of the various fungal growth was according to Raper and Fennell (1965), Haley and Callaway (1978), kwong-Chung and Bennett (1992) and Frazier and Westerhoff (1991). The methods were entirely based on the macroscopic and microscopic appearances. Macroscopically, they were provisionally identified from their gross colonial morphology and distinctive colonial colouration, while microscopically, it was based on the nature of the hyphae, conidiophore, columella, sterigmata and conidia through the needle mount techniques

3.7 COLONIAL MORPHOLOGY

This is done macroscopically by examining each culture plate under good sources of light and the description noted include

(1) Rate of growth

(2) Colour of growth

(3) Texture of growth

(4) Topography

(5) Reverse side colour

3.8 LACTOPHENOL COTTON BLUE MOUNT

Lactophenol cotton blue mount was carried out on the fungal isolates to identify exact fungi based on the conidial and hyphal arrangement. A small portion of vegetative mycelia from the culture was cut off and placed in one drop of lactophenol cotton blue on a clean slide with sterilized mounting needle. The suspension was gently teased out for separation of culture agar block from the fungal material using 2 needles. The slide was then covered with cover slip and warmed gently over Bunsen flame to allow for effective penetration and then examined using x10 and x40 objective for identification of the fungi. Arrangement, surface appearance, size, shapes, colour, distribution of fruiting bodies, spores and hypha were studied and fungi identified.

This study, following Adebajo (2000), was conducted to determine the participatory roles of isolates in the spoilage of the nuts. Fresh and healthy cotyledons were dry-milled, oven-dried at 90^0C to constant weight, mixed thoroughly and 25g portions dispensed into 250ml conical flasks. The flasks were cotton stoppered and wrapped in aluminum foil before autoclaving for 15

minutes at 121^0C twice in 24 hours. 6ml of aqueous conidial suspension (10^6ml) of 6 day old culture of a test fungus maintained on PDA slants was aseptically inoculated onto the substrate in each flask. The latter was rotated during the process to ensure even distribution of the conidia-bearing mist each experiment was replicated five times. The approximate moisture content of the substrates after inoculation was 21.2%, the incubation was at 28^0C for 30 days after which the content of each flask was oven dried at 90^0C for 48 hours, cooled in a desiccator over anhydrous $Cacl_2$ and finally weighed the difference in weight at the beginning and end at the experiment was due to fungal degradation.

3.9 DETERMINATION OF AFLATOXIN LEVELS

Sample collection

All the samples of cashew nuts which yielded *Aspergillus flavus* growth were analyzed using an immuno chromatographic test (RIDA® quick alfatoxin test strips

Sample preparation

Twenty grams (20.0g) of each composite sample was ground using a Romer series 11 Miller/Warring blender. Then 5.0g of the sample was weighted into a pre- cleaned jar followed by the addition of 10 ml of 70 to 30 (v/v) methanol water extraction solution and the jar was sealed and shaken vigorously for 3 minutes and then allowed to settle thereafter, the supernatant was filtered through a Whatman No. 1 filter paper and the filtrate collected (R-Biopharm kit)

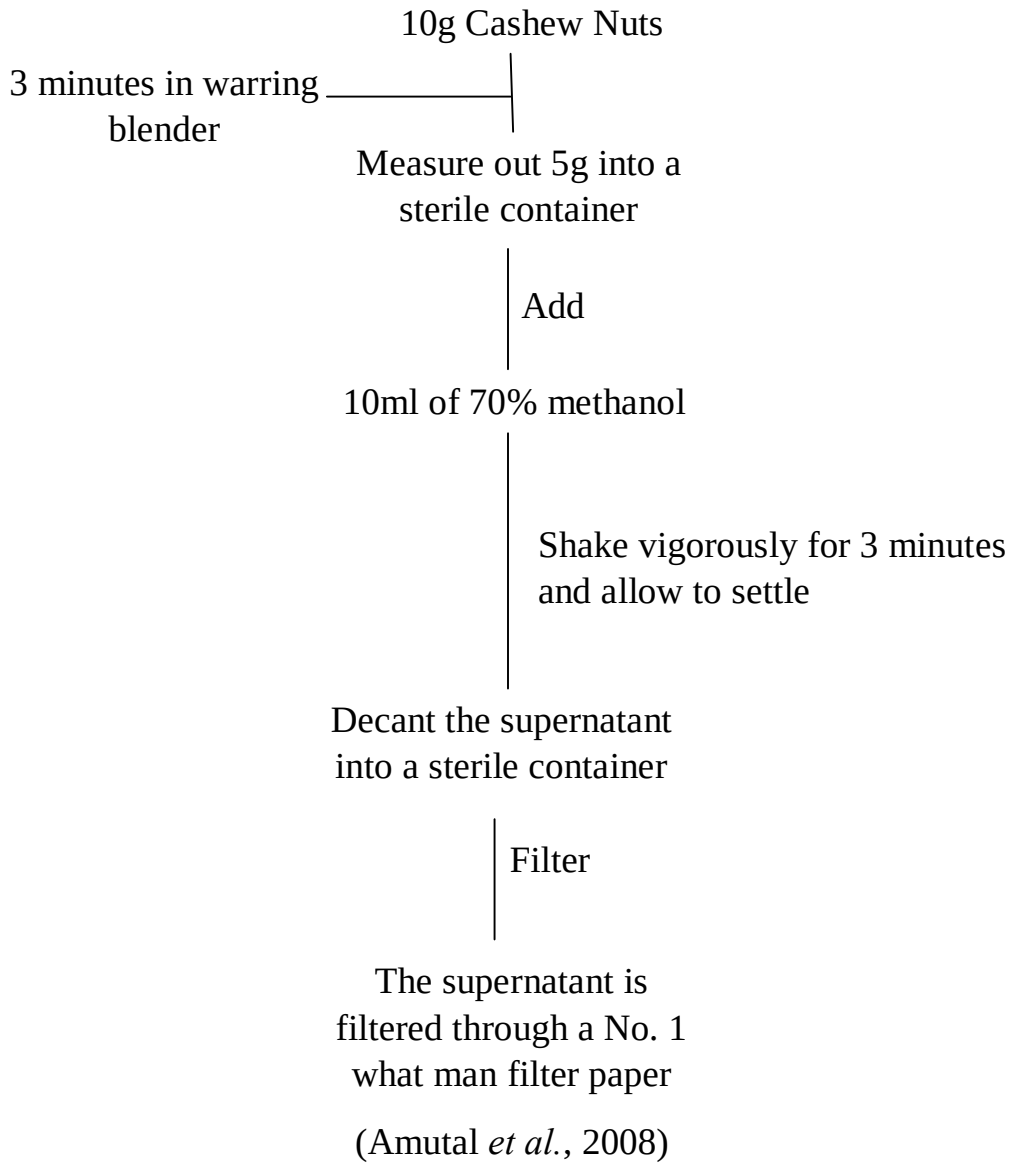


Figure 1: Extraction steps of aflatoxin from cashew nuts

3.10 ASSAY RADA ® QUICK AFLATOXIN TEST STRIPS ASSAY

RIDA® Quick aflatoxin immunochromatographic test

The Test Principle

The basis of the immune chromotographic assay in test strip formate is an antigen- antibody reaction. A specific antibody against aflatoxin recognizes the aflatoxin molecules in the samples. The results are read usually by observing the development of coloured bands. The control band is not influenced by aflatoxin in the sample and should be present in all cases in order to prove the test strip is valid.

Reagents

- Each kit contains sufficient materials for 20 determinations. Each test kits contain 20 x test kits strips. 1 x mobile solvent (2.5ml)
- 1 x evaluation card
- Methanol (70%)

Apparatus

- Laboratory miner/grinder
- Balance
- Shaker
- Filter paper, whatman no. 1
- Graduated cylinder
- 50 and 100ml pipette

3.11 SAMPLE PREPARATION

- Bring the mobile solvent to room temperature before use
- Weigh 5g of ground cashew nut sample into a suitable container

- Add 10ml methanol (70%)
- Close the tube and shake the sample vigorously for 3 ï 5 minutes
- Let the solution come to sedimentation for 3 minutes
- Filter the solution
- Prepare 100 µl of the temperate mobile solvent
- Add 50 µl of the clear supernatant, mix it and use 100 µl in the test
- Apply 100µl of the sample solution on the application area of the test strip
- Read the result after 4 , 8 or 16 minutes

Result Reading

Control band

The test is valid, if the control band is clearly visible

Negative sample

The sample is free of aflatoxin, if only the control band is clearly visible

Positive sample

The sample is contaminated with aflatoxin, if the control band is visible and the test band is also visible

3.12 DIRECT ULTRAVIOLET (UV) VIEWING:

Samples of the various cashew nuts were subjected to the bright greenish yellow fluorescence (BGYF) presumptive test under the ultraviolet (UV) light at 360nm, using the UV lamp (Shimadzu crop, Kyoto, Japan). Fluorescence indicates the presence of aflatoxins (Food and Agriculture Organization 117).

3.13 EXPERIMENTAL ANIMALS

A total of eight (8) albino wistar rats weighing 106g to 160g were obtained from the animal house of the University of Nigeria Teaching Hospital (UNTH). They were brought at age of 6 weeks and were housed under standard conditions of temperature (22 ± 3^0C) and 12 hours light and 12 hours dark cycle. The animals were housed in two groups and were provided with water and standard pellets (guinea feed®) ad libitum. The period of acclimatization was 3 days before onset of the experimental study

The animals were divided into two (2) groups; A and B. The group A (control) contained 4 mice in a cage and the group B (test) contained four mice in the cage making a total of 8 mice. The control group (A) were fed with only standard pellets while the test group (B)are fed with 50:50 ratio of standard pellets and cashew nuts which yielded positive aflatoxin detection depending on the weight of the mice for 10 days. Animals were monitored daily for 10 days and examined for vital signs and death.

3.14 WEIGHT OF THE LABORATORY ANIMAL

Group A Wistar Rats (Control)

- 110g
- 106g
- 116g
- 110g

Group B Wistar Rats (Test)

- 127g
- 128g

- 168g
- 169g

3.15 BLOOD SAMPLE COLLECTION FROM THE WISTAR RATS

Blood samples were collected from the mice after ten (10) days and placed into a sterile bottle free from anticoagulants. These samples were taken to laboratory for electrolytes/urea/creatinine and liver function tests.

3.16 BIOCHEMICAL TEST

(1) Electrolytes test

(2) Urea

(3) Creatinine

(4) Liver function test

3.16.1 ELECTROLYTES TEST

The blood samples were taken to the laboratory for electrolyte analysis using Roche Diagnostics AVL 9180 series Electrolyte Analyzer. The analyzer is fully automated ensuring precision and conformity to even the most stringent regulatory requirements. The electrolyte tests include;

- Sodium
- Potassium
- Chloride
- Calcium

3.16.2 LIVER FUNCTION TEST

Liver function tests are a group of blood tests that detect inflammation and damage to the liver. They also check how well the liver is working. Liver function tests include:

- Alkaline phosphatase
- Alanine aminotransferase
- Aspartate aminotransferase
- Bilirubin ;direct and total

3.16.3 CREATININE TEST

The creatinine blood test is used to assess kidney function. It is a waste product produced by muscles and put out through the kidneys. This means that the normal levels depend on how much muscle one has and this can cause some problems in spotting kidney disease. The analysis was carried out using Roche Diagnostics AVL 9180 series Electrolyte Analyzer.

3.16.4 UREA TEST

The urea test is a common blood test used to assess kidney function. Urea is a small molecule that is produced in the liver that you have eaten. It is put out by the kidneys. Urea is still a very useful test when used together with creatinine to measure how well kidney is working to clear waste products The test was done using Roche Diagnostics AVL 9180 series Electrolyte Analyzer.

3.17 AUTOPSY

The wistar rats were sacrificed and autopsied at death. Portion of the liver and kidney were fixed in 10% formal saline for histological studies.

3.17.1 HISTOLOGICAL PROCESSING

The fixed portions of the liver and kidney were prepared with haematoxylin and eosin and studied under the light microscope. The steps involved in tissue preparation according to Gartner

and Hiatt (2001) are fixation, dehydration and clearing, embedding in a suitable medium, sectioning into slices to permit viewing by trans-illumination, mounting onto a surface for ease of handling and standing so that the various tissue and cell components may be differentiated.

- **Fixation**

Fixation is the treatment of the tissue with chemical agents that not only retard the alterations of tissue subsequent to death but also maintain itôs normal architecture.

- **Dehydration and Clearing**

The water in the tissue is removed with graded series of alcohol baths, beginning with 50% alcohol and progressing in graded steps to 100% alcohol. This is called dehydration.

- **Clearing:**

The tissue is treated with xylene; a chemical that is miscible with melted paraffin. This helps to make the tissue transparent.

- **Embedding:**

This is to distinguish overlapping cells in a tissue and extracellular matrix from one another. The embedding medium is paraffin. It is done by placing the tissue in a suitable container of melted paraffin until the tissue is completely infiltrated. Once the tissue is impregnated with paraffin, it is placed into a small receptacle, covered with melted paraffin and allowed to harden, forming a paraffin blook containing the tissue.

- **Sectioning:**

After the blocks of tissue are trimmed of excess embedding material, they are mounted for sectioning using a microtome. For light microscope, the thickness of each section is about 5 ï 10 µm.

- **Mounting and Staining**

Paraffin section are mounted or placed on glass slides and then stained with water soluble stains (haematoxylene and eosin for this study) that permit differentiation of the various cellular components. Heamatoxylin colours the acidic components of the cell DNA and RNA while Eosin is an acid dye that stains the basic components of the cell pinkish colour. Many cytoplasmic constituents have a basic pH and stains pink. Stained slides were also photographed with photomicron camera, developed and printed to magnify abnormalities as may be seen in the portion of organs processed.

3.18 STATISTICAL ANALYSIS

All data generated from the study were anlaysed using the Statistical Package for Social Sciences (SPSS) versions 10.

CHAPTER FOUR

RESULTS

From the 152 samples of cashew nuts sampled (table 4.1), *Aspergillus flavus* group showed the highest incidence 83 (35.4%) followed by *Aspergillus niger* 81(34.6%), *Mucor* species 23(9.8%), *Rhizopus* species 17(7.3%), *Rhodotorula* species 11(4.7%), *Aspergillus fumigatus* 12(5.1%), *Candida* species 4(1.7%), *Aspergillus nidulans* 2 (0.4%) and *Geotrichum* species 1(0.4%). Altogther, nine (9) species of fungi were isolated at varying levels from the cashew nuts, and a total of 6 genera.

Table 4.1: General Distribution of Fungal Isolates in surface Disinfected Cashew Nuts

Fungal Isolates	Frequency
Aspergillus flavus	83
Aspergillus niger	81
Mucor species	23
Rhizopus species	17
Aspergillus fumigatus	12
Rhodotorula species	11
Candida species	4
Aspergillus nidulans	2
Geotrichum species	1
Total	**234**

The distribution of fungal isolates in the two groups of surface disinfected cashew nuts is shown in Table 4.2. Of the 152 samples of cashew nuts investigated, 62 samples were exposed cashew nuts while 90 samples were unexposed. *Aspergillus flavus* isolated from unexposed group ranked highest 61 (25.6%) followed by *Aspergillus niger 49* (20.9%). *Geotrichum* species was not isolated in the exposed group while 1 (0.4%) was isolated from the unexposed group. There was no significant difference between the distribution of the fungal isolates in relation to their groups (exposed and unexposed).

Table 4.2: General Distribution of Fungal Isolates In Exposed And Unexposed Cashew Nuts

Fungal isolates	Exposed	Unexposed
Aspergillus flavus	22	61
Aspergillus niger	32	49
Aspergillus fumigatus	8	4
Mucor species	12	11
Rhizopus species	7	10
Rhodotorula species	3	8
Aspergillus nidulans	1	1
Geotrichum species	Nil	1
Candida species	1	3
Total	**86**	**148**

The distribution of fungal contamination of exposed cashew nuts is shown in Table 4.3 Of the 62 samples of exposed cashew nuts, *Aspergillus niger* showed highest incidence 32(37.2%), followed by *Aspergillus flavus* 22(25.5%), *Mucor* species 12 (13.9%), *Aspergillus famigatus* 8(9.3%), *Rhizopus* species 7(8.1%), *Rhodotorula* species 3(3.4%), *Aspergillus nidulans* 1(1.1%) and *Candida* species 1(1.1%).

Table 4.3: Distribution of Fungal Contamination Of Exposed Cashew Nuts

Fungal Isolates	Frequency
Aspergillus flavus	22
Aspergillus niger	32
Mucor species	12
Aspergillus fumigatus	8
Rhizopus species	7
Rhodotorula species	3
Aspergillus nidulans	1
Candida species	1
Geotrichum species	Nil
Total	**86**

The distribution of fungi contamination of unexposed cashew nuts is shown in Table 4.4. Out of the 90 samples investigated, *Aspergillus flavus* ranked highest 61 (41.2%), *Aspergillus niger* 49(33.1%), *Mucor* species 11(7.4%), *Rhizopus* species 10 (6.7%), *Rhodotorula* species 8(5.4%), *Aspergillus fumigatus* 4(2.7%), *Candida* species 3(2.0%), *Aspergillus nidulans* 1 (0.6%) and *Geotrichum* species *1* (0.6%).

Table 4.4: Distribution of Fungal Contamination Of Unexposed Cashew Nuts

Fungal Isolates	Frequency
Aspergillus flavus	61
Aspergillus niger	49
Mucor species	11
Rhizopus species	10
Rhodotorula species	8
Aspergillus fumigatus	4
Candida species	3
Aspergillus nidulans	1
Geotrichum species	1
Total	**148**

Distribution of *Aspergillus flavus* mixed growth is shown in Table 4.5

Out of the 62 samples of exposed cashew nuts investigated only *Aspergillus flavus, Aspergillus niger, Mucor* species*, Aspergillus fumigatus* and *Rhodotorula* species yielded mixed growth. *Aspergillus flavus and Aspergillus niger* showed the highest incidence 9 (64.2%) while *Aspergillus flavus and Mucor* species *sh*owed the least growth incidence 1 (14.2%).

Of the 90 samples of unexposed cashew nuts investigated only *Aspergillus flavus, Aspergillus niger, Mucor* species*, Aspergillus fumigatus, Aspergillus nidulans* and *Rhodotorula* species *yi*elded mixed growth. *Aspergillus flavus* + *Aspergillus niger* ranked highest 29(63%) while *Aspergillus flavus* + *Aspergillus nidulans* and *Aspergillus flavus* + *Aspergillus fumigatus* showed least incidence. The difference was statistically significant (P> 0.05).

Table .4.5: Distribution of Aspergillus Flavus Mixed Growth with other fungal Isolates

Fungal Isolates	Frequency
Aspergillus flavus + *Aspergillus niger*	29
Aspergillus flavus + *Rhodotorula* species	6
Aspergillus flavus + *Rhizopus* species	5
Aspergillus flavus + *Mucor* species	4
Aspergillus flavus + *Aspergillus nidulans*	1
Aspergillus flavus + *Aspergillus fumigatus*	1
Total	**46**

Table 4.6 shows the general distribution of *Aspergillus flavus* encountered in the cashew nuts according to their categories. Unexposed cashew nuts yielded more strains of *Aspergillus flavus* 61 (41.2%) than the exposed cashew nuts which yielded 22 (25.6%) of *Aspergillus flavus* strain.

Table 4.6 General Distribution of *Aspergillus flavus* Encountered in the study

Fungal isolates	Exposed	Unexposed
Aspergillus flavus	22	61
Total	**22**	**61**

Table 4.7 shows that out of the total of 83 *Aspergillus flavus* specie encountered from various cashew nuts, only 18 were found to be toxigenic using the rida ® quick aflatoxin immunchromatographischer test kit.

Table 4.7 General Distribution of aflatoxins from sampled cashew nuts

Cashews nuts	Results	Growth
UE 76	10ppb	Asperillgus *flavus* + *Aspergillus fumigatus*
UE 150	24 ppb	*Aspergillus flavus*
UE 72	115 ppb	*Aspergillus flavus*
UE 89	96 ppb	*Aspergillus flavus* + *Mucor*
UE 120	20 ppb	Asperillgus flavus
UE 118	15 ppb	*Aspergillus flavus*
UE 51	20 ppb	*Aspergillus flavus*
UE 58	96 ppb	*Aspergillus flavus* + *mucor* species
UE 55	Negative	Asperillgus flavus + *Aspergillus niger*
UE 53	Negative	*Aspergillus flavus* + *Aspergillus niger*
UE 46	4ppb	*Aspergillus flavus*+ *Rhizopus*
UE76	20 ppb	*Aspergillus flavus* + *Aspergillus flavus*
UE91	Ò4 ppb	*Asperillgus niger* + *Aspergillus flavus*
UE128	Negative	*Aspergillus flavus* +*Rhodotorula* species
UE70	6 ppb	*Aspergillus flavus*+ *Aspergillus niger*
UE13	10 ppb	*Aspergillus flavus*
UE72	10 ppb	Asperillgus flavus + *Aspergillus niger*
UE133	105 ppb	*Aspergillus flavus*
UE150	24 ppb	*Aspergillus flavus*
UE132	Negative	*Aspergillus flavus* + *Aspergillus niger*
UE131	Negative	*Asperillgus flavus* +*Rhodotorula* species
UE144	Negative	*Aspergillus flavus*+*Rhodotorula* species
E125	90 ppb	*Aspergillus flavus*
E90	Negative	*Aspergillus flavus*+ *Rhizopus* species
E52	Negative	*Aspergillus flavus* + *Aspergillus niger*
E108	18 ppb	*Aspergillus flavus*+ *Aspergillus niger*

Table 4.8 Weight of Laboratory Animals After 10 Days of Feeding

Group A Wistar Rats (Control)

- 110g
- 107g
- 118g
- 111g

Group B Wistar Rats (Test)

- 125g
- 126g
- 166g
- 157g

Table 4.9 shows the biochemical characteristics of the test and control wistar rats. It includes the liver function test and renal function tests. The renal function test mean value for the test albino wistar rats are urea (30.8± 2.8), Na (142.25 ± 1.65), potassium (7.12± 0.19), chloride (112.50 ± 1.322) ($P > 0.05$). The calcium mean value (112.50± 1.32) and creatinine mean value (0.50 ±0.05) are statistically significant ($P<0.05$). The liver function test mean value for the albino test rats are ALP (124.24 ± 17.77), ALT (23.12 ± 4.74), AST (39.92 ± 4.44) which were not statistically significant ($P>0.05$).

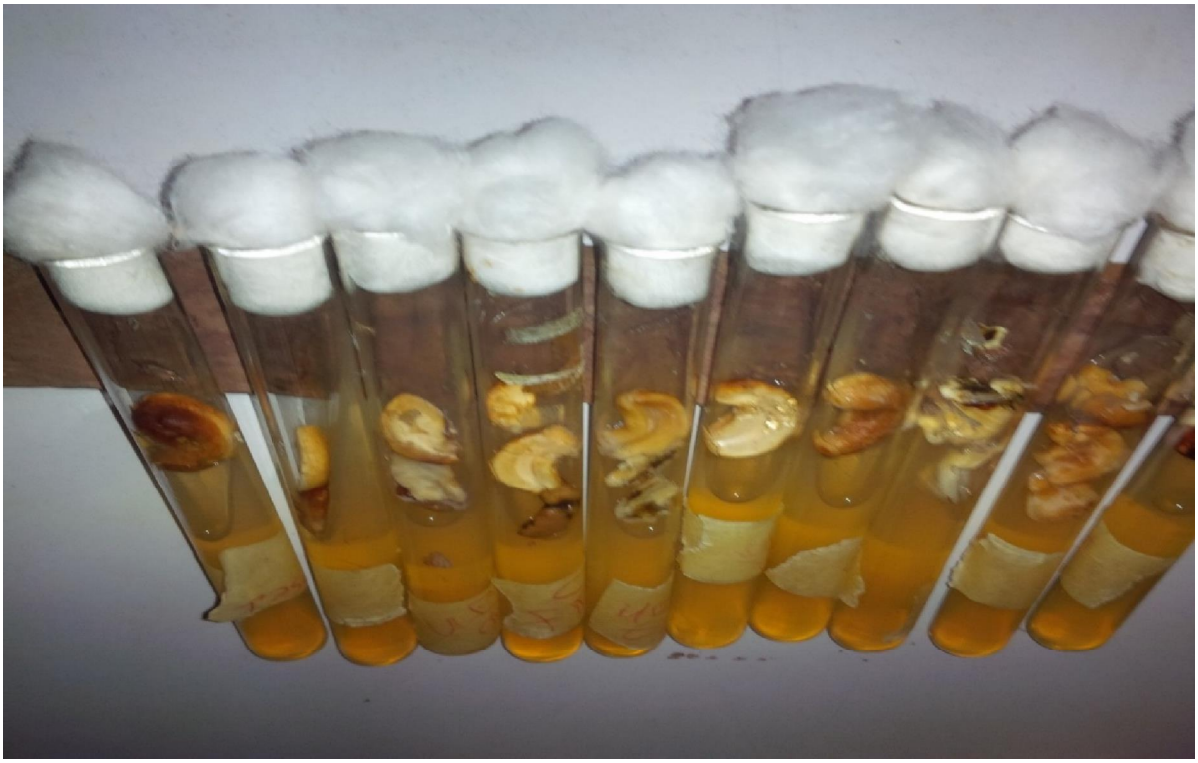


Plate 4.1: Sabouraud agar slants showing culture of cashew nuts

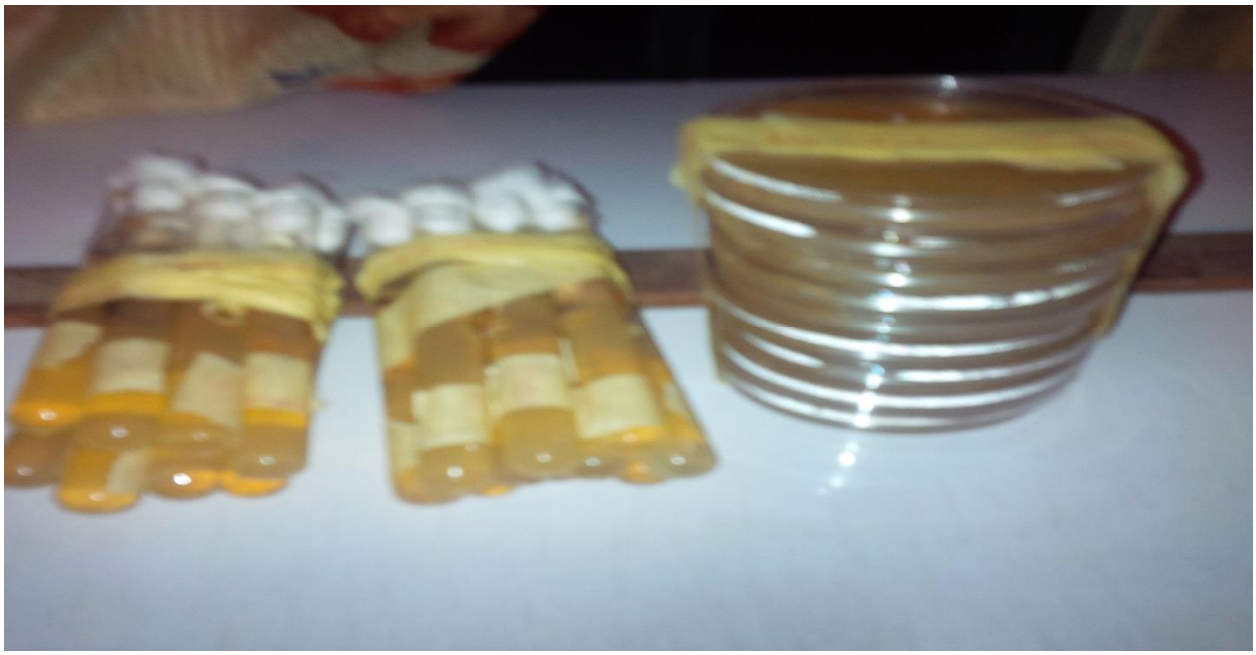


Plate 4.2: Sabouraud agar slant and plates showing culture of cashew nuts

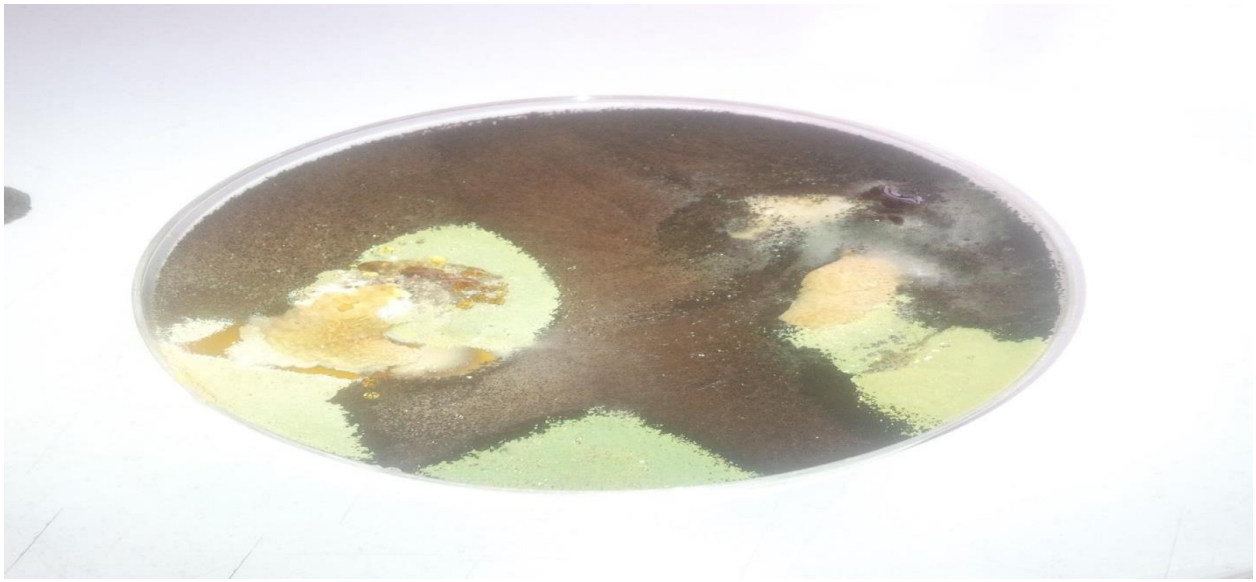


Plate 4.3: Sabouraud agar plate showing mixed growth of *Aspergillus flavus* and *Aspergillus niger*

Plate 4.4: Sabouraud dextrose agar plate showing mixed growth of *Aspergillus flavus* and *Rhodotorula* species

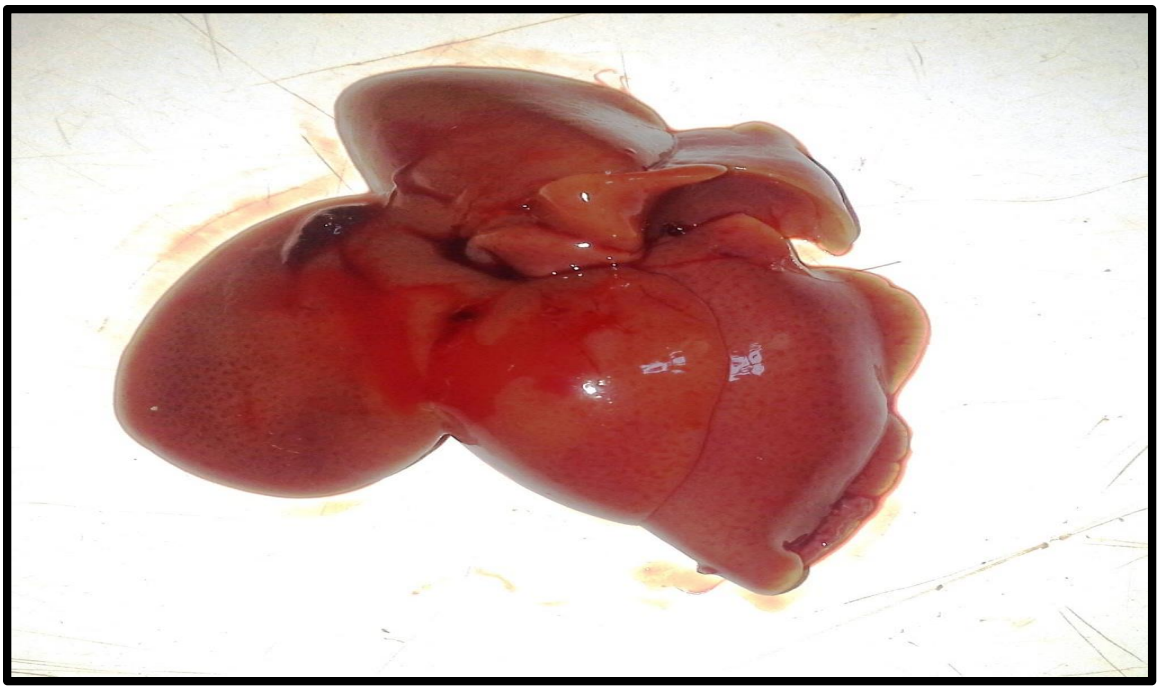


Plate 4.5: Photomacrograph showing normal gross morphology of the Liver of rat in Control group.

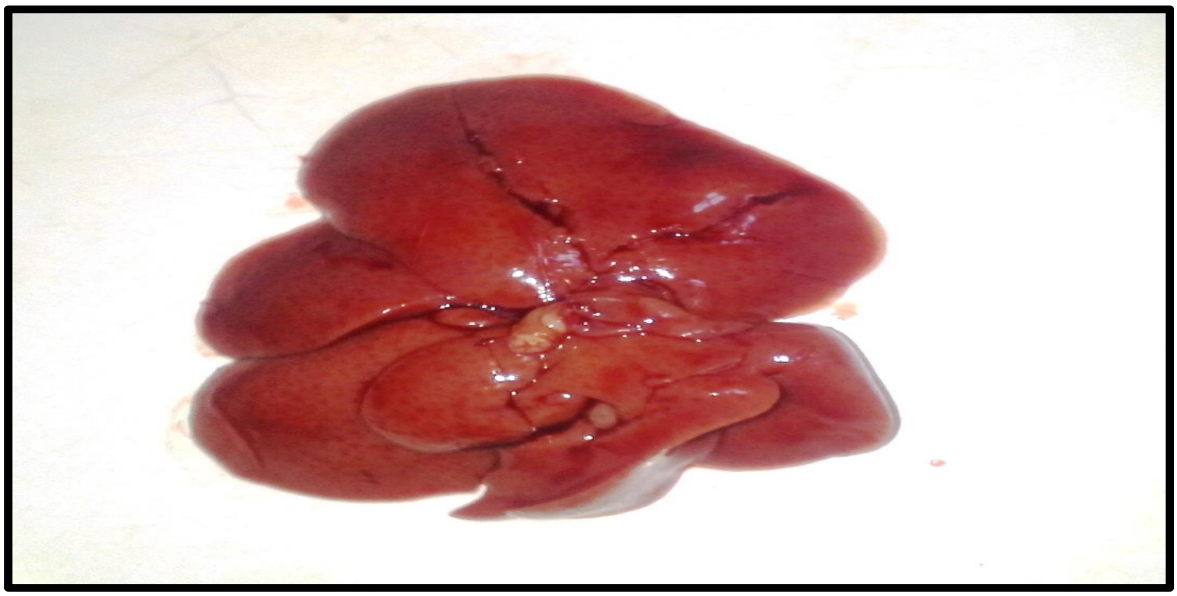


Plate 4.6; Photomacrograph showing the gross morphology of the liver of rat fed with Cashew nuts. No obvious macroscopical change is observed.

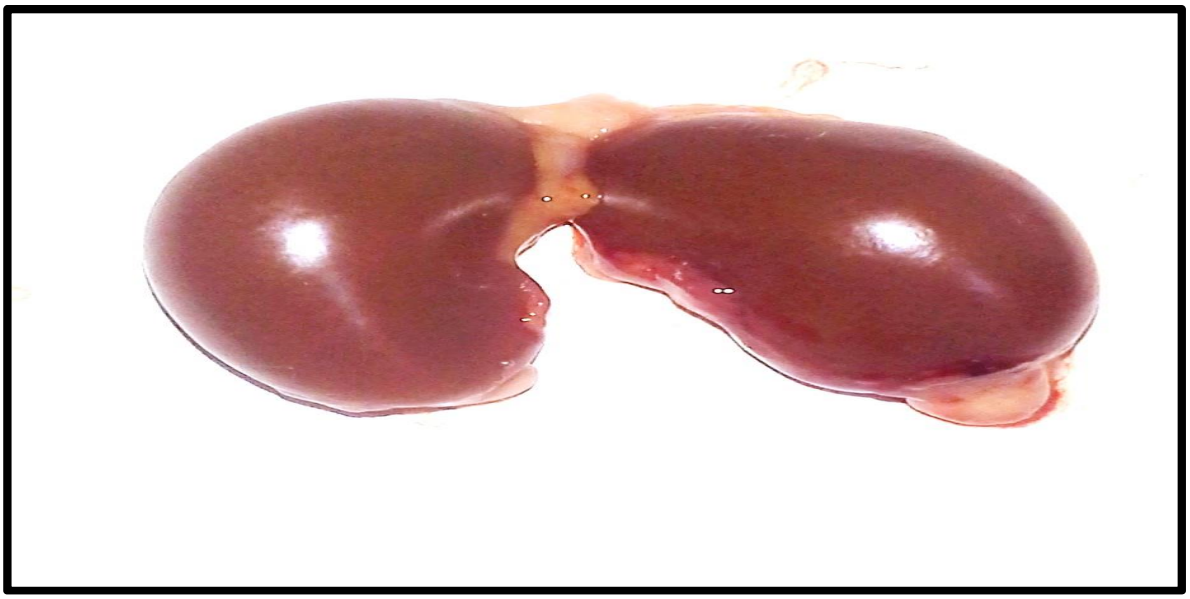


Plate 4.7: Photomacrograph showing normal gross morphology of the kidney of rat in Control group.

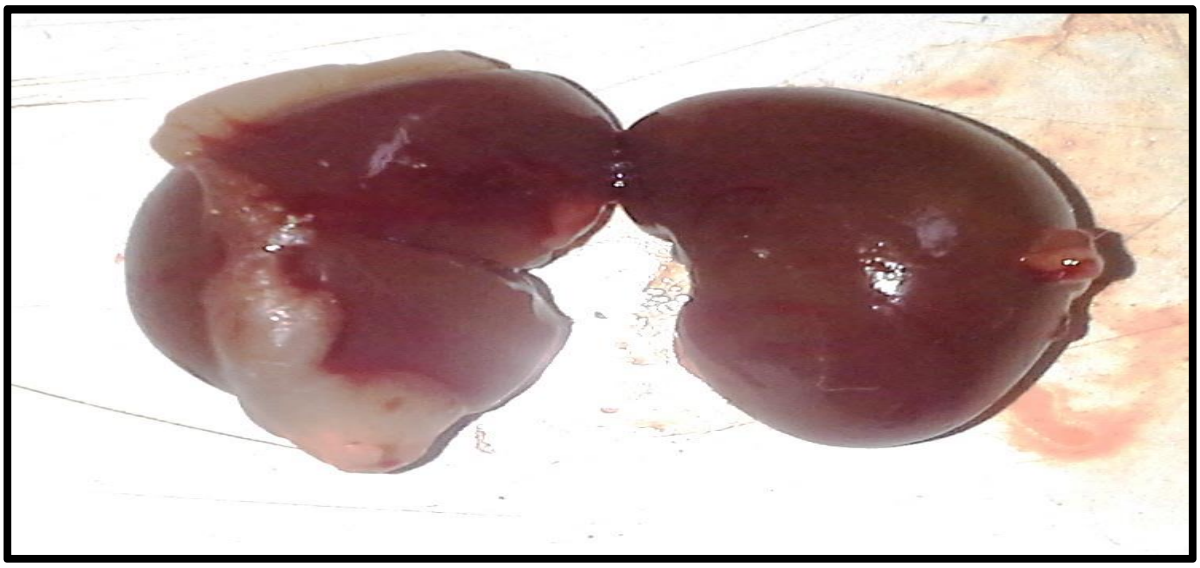


Plate 4.8: Photomacrograph of kidney from rat fed with cashew nuts showing no obvious pathological change macroscopically.

HISTOPATHOLOGICAL EXAMINATION OF THE LIVER

Histopathological examinations of liver in control animals liver section (Plate 4.9) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and central view. No obvious histological changes could be noticed in the liver. In test animals (Plate 4.10), histological evaluation of the effect of aflatoxin showed marked distortion of hepatic architecture; marked widening of sinusoids, central canal rupture, degenerating hepatocytes and inflammatory cellular infiltrations within the sinusoids.

HISTOPATHOLOGY OF THE LIVER

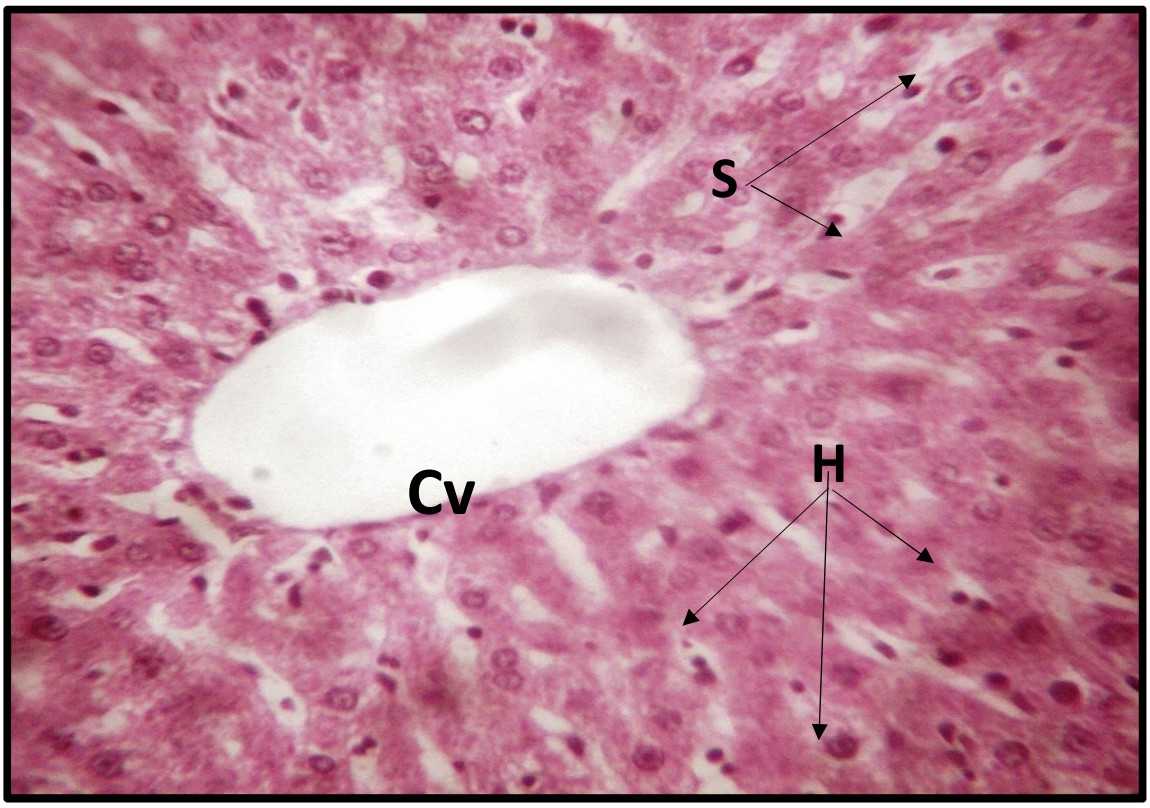


Plate 4.9: Light photomicrograph of Control rat (Group A) Liver section showing normal histomorphology. The central vein (Cv), hepatocytes (H), sinusoids (S) and portal tract (not shown) are normal. [Stain: H&E/Mag: x400]

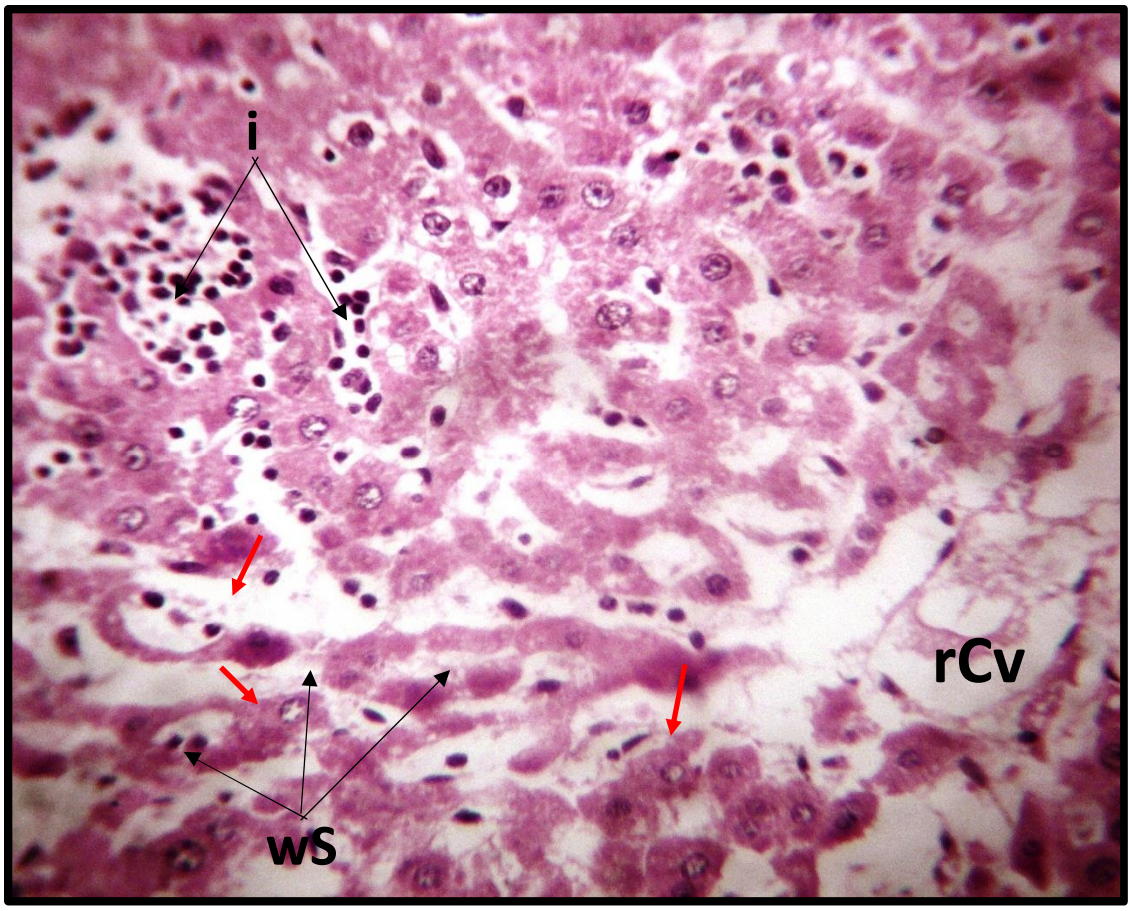


Plate 4.10: Light photomicrograph of liver section from rat fed with 50% cashew nut diet (Group B) showing marked widening of the sinusoids (wS), central canal rupture (rCv), degenerating hepatocytes (red arrows) and inflammatory cellular infiltration especially within the sinusoids (i). [Stain: H&E/Mag: - x400].

HISTPATHOLOGICAL EXAMINATIONS OF KIDNEY

Microscopic examination of kidney section in control group stained with H & E showed normal appearance of glomeruli, bowman capsule, proximal and distal tubules as demonstrated in Plate 4.11: [A]. Administration of aflatoxin contaminated cashew nuts to the rats also showed normal architecture of the rat kidney in Plate 4.11: [B].Examination of kidney tissue revealed no evidence of renal tubular injury after 10 days of feeding with aflatoxin contaminated cashew nuts.

HISTOPATHOLOGY OF THE KIDNEY

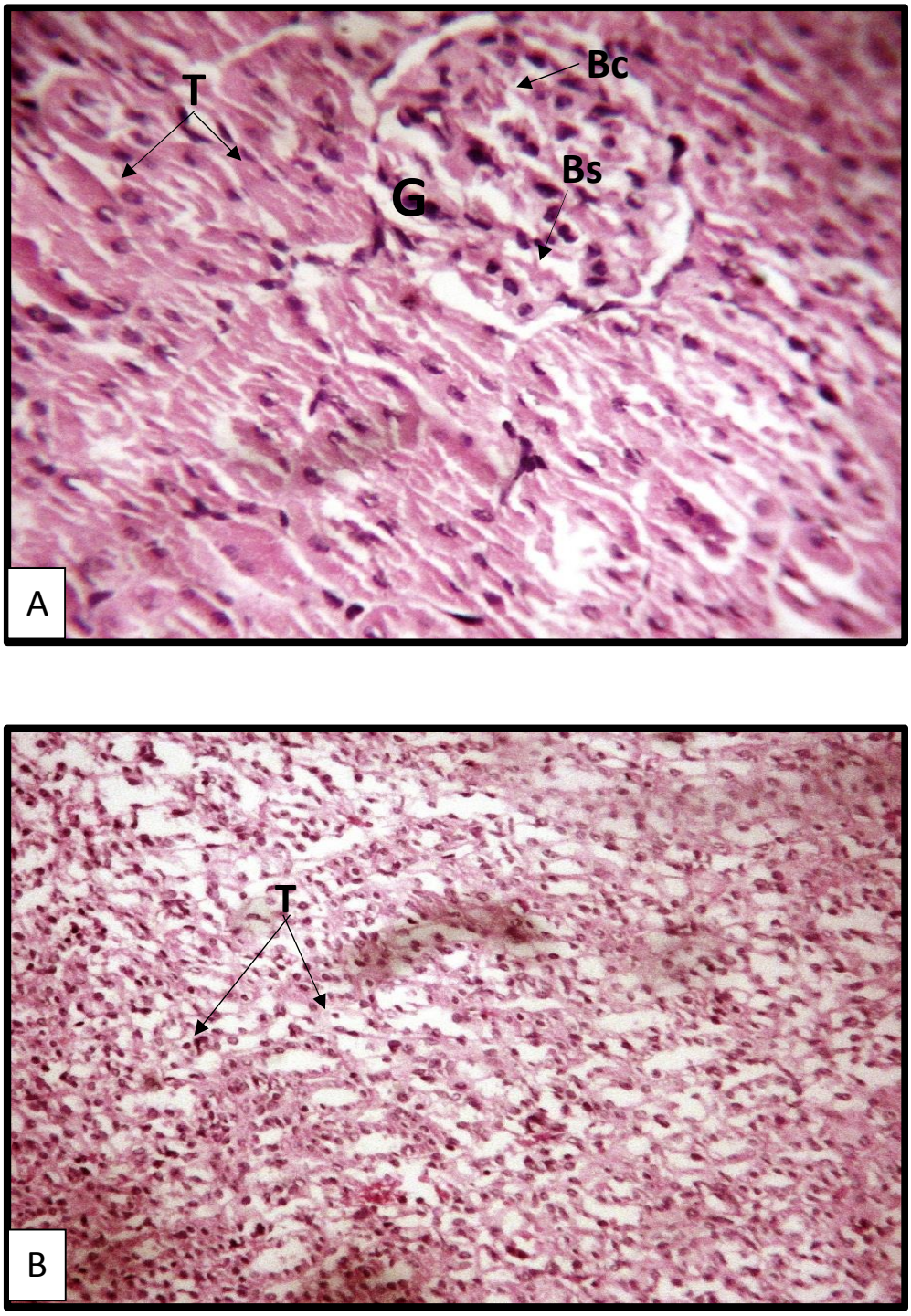


Plate 4.11 [A & B]: Light photomicrographs of control rat (Group A) kidney section showing normal cortical (A) and medullary (B) portions of the renal tissue. The features observed are normal glomerulus (G), tubules (T), Bowmanôs capsule (Bc) and space (Bs). [Stain: H&E/Mag: A-x200; B-x100]

HISTOPATHOLOGY OF THE KIDNEY

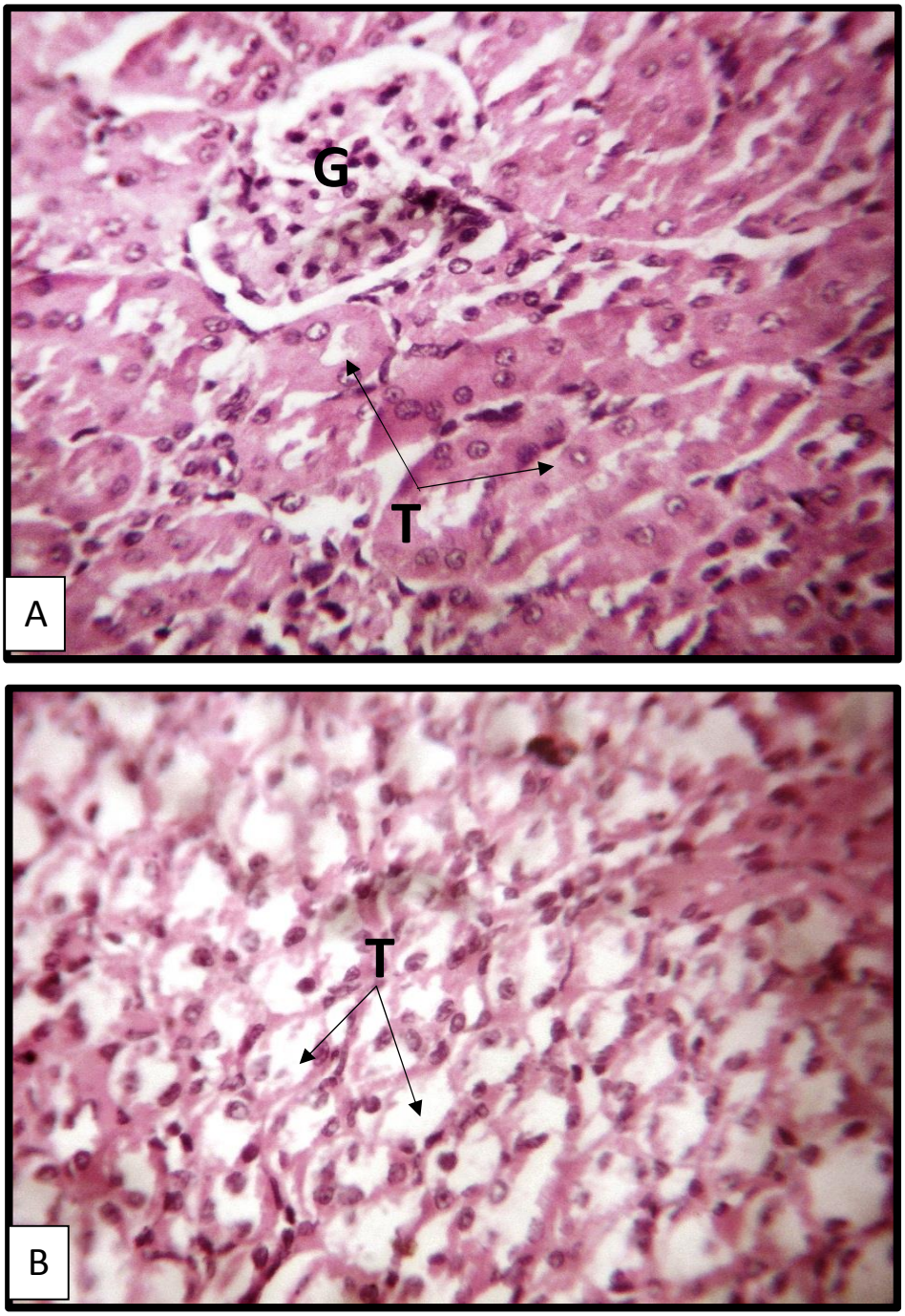


Plate 4.12 [A & B]: Light photomicrographs of kidney sections from rat fed with 50% cashew nut diet (Group B) showing no observable abnormality with features in the cortical and medullary portions of the renal tissue. [Stain: H&E/Mag: - x200].

CHAPTER FIVE

DISCUSSION

Worldwide, cashew nuts are an esteemed and highly priced food delicacy. The nuts are predisposed to mould contamination due to crude method of processing and packaging leading to aflatoxin contamination. Aflatoxins and especially aflatoxin B_1 are associated with both toxicity and carcingogenicity in human and animal population (International Agency for Research on Cancer, 1982). Diseases caused by aflatoxin consumption are loosely called aflatoxicosis. Acute aflatoxicosis results in death. Chronic aflatoxicosis results in cancer, immune suppression and other slow pathological conditions (Hsieh, 1988). The liver is the primary target organ with liver damage occurring. Thousands of studies on aflatoxins have been done mostly on laboratory models (Butler, 1969; Cullen and Newberne, 1994; Eaton Groopman, 1994).

A total of 152 samples of exposed and unexposed cashew nuts were analysed. Table 4.1 shows that *Aspergillus* species accounted for a higher prevalence than non- *Aspergillus* species. This result agrees with the work done by Nyrahakizimana *et al.* (2013) in Turkey who discovered that the genus *Aspergillus* specie was the major fungus that affect nuts. In this study, the results of isolation indicated that the *Aspergillus niger* and *Aspergillus flavus* were the fungi present in all classes of nuts.

The results indicate that the 152 samples of exposed and unexposed cashew nuts analysed were subject to mould contamination with dominance of *Aspergillus flavus* group. Out of the nine mould species isolated. *Aspergillus flavus 8*3 (35.4%) ranked highest being followed closely by *Aspergillus niger* 81(34.6%), *Mucor* specie 23(9.8%), *Rhizopus* specie 17 (7.3%), *Rhodotorula* specie 11 (4.7%), *Aspergillus fumigatus* 12 (6.1%), *Candida* specie 4 (1.7%), *Aspergillus*

nidulans 2 (0.8%) and *Geotrichum* specie 1 (0.4%). This work agrees with the work done by Nyirahakizimana *et al.* (2013) in Turkey where *Aspergillus flavus* and *Aspergillus niger* were the most fungi isolated. It also agrees with work done by Onyemelukwe (1999), in parts of Enugu where samples of 18 Nigerian staple foods analyzed are subject to mould contamination with dominance of the *Aspergillus flavus* group. Out of the sixteen mould groups isolated, *Aspergillus flavus* ranked highest 34.8% being followed closely by *Penicillium* specie (16.8%), *Aspergillus niger* (12.0%), *Aspergillus fumigatus* (8.6%) and *Fusarium* specie (7.8%). This result however, disgrees with the work done by Adebajo and Diyaolu (2003) in Lagos, Nigeria where a total of 32 samples were analyzed. Fourteen fungi belonging to five genera were recovered at varying levels from the cashew nuts with a dominance of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Rhizopus nigricans and Mucor* specie.

This result also disagrees with the work done by Ismail *et al*., (2014) in Instabul where 50 samples of cashew nuts where analysed. 14 out of the 50 samples yielded *Aspergillus flavus* growth. The incidence and level of *Aspergillus flavus* and aflatoxins found in this study were relatively low. This work also contradicts the work of Abdel ï Gawad and Zohzi (1993), whose result indicated that *Cladosporium specie* showed highest incidence (70%). Some of the species of *Aspergillus* (Table 4.1) associated with easy nuts are known to have strains that produce toxic metabolites. (Bamburig *et al*., 1969; Cole and Cox,1981). Thus they pose a potential harzard to consumerôs health. The conditions generally known to influence the production of aflatoxin in foods include high temperature, moisture, unseasonal rains, presence of toxigenic mould, a suitable substrate for the growth of the mould and an environment conducive for the toxin production (Betina, 1984).

Table 4.2 shows incidence of fungi isolates in relation to their group viz; exposed and exposed. The result indicates that the exposed group yielded 86 fungal isolates while the unexposed group yielded 148 fungal isolates. However, fungal isolates from unexposed group were greater in number than the fungal isolates from exposed group. There was statistical difference between the two groups ($P<0.05$).

Table 4.3 shows that out of the 62 samples of exposed cashew nuts, *Aspergillus niger* ranked highest 32 (37.2%) followed by *Aspergillus flavus* 22 (25.5%) while from the unexposed group, *Aspergillus flavus* showed that highest incidence 61(41.2%) followed by *Aspergillus niger* 49(33.1%). This result agrees with the work done by Adebajo and Diyaolu (2003) in Lagos where a total of 32 samples were analysed, *Aspergillus niger* ranked highest followed by *Aspergillus flavus, Aspergillus fumigatus, Rhizopus* specie *and Mucor* specie. Ababutain (2013) stated that the temperature and relative humidity was important factors affecting the growth *Aspergillus niger*. In a study conducted in Brazil, the results showed that *Aspergillus niger* was the most contaminated fungus in Brazilian pistachios.

Table 4.4 shows that out of the 92 samples of unexposed cashew nuts investigated, *Aspergillus flavus* ranked highest 61(41.2%) followed by *Aspergillus niger* 49(33.1%), *Mucor* specie 11(7.4%), *Rhizopus* specie 10(6.7%), *Rhodotorula specie* 8(5.4%), *Aspergillus fumigatus* 4(2.7%), *Candida* specie 1(0.6%), and *Geotrichum* specie 1(0.6%). This agrees with the work done by Onyemelukwe (1999) which also recorded highest incidence of *Aspergillus flavus* and disagrees with the work done by Adebajo and Diyaolu (2003), which recovered higher number of *Aspergillus niger.* Some of these species, especially of *Aspergillus flavus* associated with the cashew nuts are known to have strains that produce toxic metabolities (Bamburg *et al*., 1969;

Cole and Cix, 1981).This result agrees with the work done by Abdel-Gawad and Zohri (1993) who found *Aspergillus flavus* in cashew nuts, hazel nuts and pistachio nuts.

Thus, they pose a potential hazard to consumerôs health. The conditions generally known to influence the production of mycotoxins in foods and allied agricultural products include toxigenic mould, a substrate for the growth of the mould and a condusive environment (Betina, 1984). Thus, the high temperature (25 to 33^0) and relative humidities (Ó 80%) prevalent in Southern Nigeria Ogundero (1987) suggest that elaboration of mycotoxin in poorly packaged and stored cashew nuts samples is to be expected. Specifications for fungal contamination in packaged nuts are usually related to the nature of the product and the packaging materials. According to Lacey (1989), most fungal contamination occur during drying and develops during storage with species of *Aspergillus* being largely dominant. The findings indicate that temperature, relative humidity and moisture content play important roles in mould contamination. Thus, *Aspergillus flavus* group dominate in the hot dry seasons and raining seasons. Similar pattern of seasonality in distribution have been recorded in other tropical parts of the world (Mishra and Daradhiyar, 1991). The warm rainy seasons favours the proliferation of *Aspergillus flavus* and other moulds (Clacey, 1989; Moss 1989).

In Nigeria, however, peasant processing and packaging method predisposes the cashew nuts to mould contamination especially during storage and hawking of the nuts which are usually stored in jute bags and hawked in hand -knotted thin polyethylene bags.

Table 4.5 shows that out of the 152 samples of exposed and unexposed cashew nuts analysed, only *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Rhizopus* specie*, Mucor* specie*, Rhodotorula* specie*, and Aspergillus nidulans y*ielded mixed growth. The mixed growth of

Aspergillus flavus + *Aspergillus niger* ranked highest 29 followed by *Aspergillus flavus* + *Rhodotorula specie* with *Aspergillus flavus vs Aspergillus nidulans 1* being the least isolated.

Table 4.6 shows the general distribution of *Aspergillus flavus* encountered in the cashew nuts according to their categories. Unexposed cashew nuts yielded more strains of *Aspergillus flavus* 61 (74.4%) than the exposed cashew nuts which yielded 22 (25.6%). This is attributed to warmth and temperature provided by the thin-polyethylene bags used in packaging the unexposed cashew nuts.

Table 4.7 shows the general distribution of aflatoxins isolated from mono and mixed fungal growth. From this study, we discovered that mixed growth of *Aspergillus flavus* and *Aspergillus niger* inhibited aflatoxin production. Aflatoxins were completely reduced ($P < 0.05$) in cashew nuts. This finding is consistent with many previous reports. Other works have shown experimentally that interactions of *Aspergillus flavus* with other moulds can dramatically influence aflatoxin production and showed the inhibitory effects of *Aspergillus niger* for toxin production (Faraq *et al.*, 1993; Moss, 1996).

We discovered that *Aspergillus niger* in mixed growth with *Aspergillus flavus* on the cashew nuts did not allow the *Aspergillus flavus* to grow well and It also degraded the toxins immediately upon formation or converted them into a modified product with an entirely different RF value from aflatoxins and so could not be detected by the routine methods in this work. Hill *et al.* (1983) found that *Aspergillus niger* can inhibit Aflatoxin production in groundnut, contaminated with *Aspergillus flavus*.

The experiment also shows that mixed growth of *Aspergillus flavus* and *Rhizopus* species inhibits aflatoxin production in cashew nuts. *Rhizopus nigricans* was found to inhibit growth of

Aspergillus flavus by Weck bach and Marth (1977). Zhu *et al*. (1989) were able to decrease hyperplastic and pathological enzymic foci in AFB_1 induced hepatocelluar carcinoma in Wistar rats fed with *Rhizopus* specie mixed with rations. *Rhizopus* s*pecie*have been shown by several workers to be capable of degrading aflatoxins and reducing their inherent toxicity and potential mutagencity (Bol and Smith, 1990; Knot *et al.,* 1990).

Mixed growth of *Aspergillus flavus* and *Mucor* specie increased aflatoxin production. This agrees with the report that *Mucor* specie slightly increased aflatoxin levels in white cowpea while having no effects in bread fruits, acha grains and Ugbawka rice (Onyemulukwe, 1999). *Aspergillus flavus* in mixed growth with *Aspergillus fumigatus* shows that both mould might have similar mechanism of action. Lacey (1989) studying interactions of organism observed that most competitve species to *Aspergillus flavus* included *Penicillium* specie*, Aspergillus fumigatus* and *Aspergillus nidulans* each giving high indices of dominance against *Aspergillus flavus* in his experiments. Dual mixed growth of *Aspergillus flavus* and *Rhodotorula* species show that there was an inhibitory effect. The mixed growth shows that there was an intense coral pigmentation from *Rhodotorula* species with very slight sporulation by the *Aspergillus flavus*. It may be possible that the carbon sources for the aflatoxin production by *Aspergillus flavus* in this dual culture with *Rhodotorula* species may have been directed towards pigment production. It is likely that *Rhodotorula* specie*s* produce diffusible compounds which inhibit aflatoxin biosynthesis of *Aspergillus flavus*. *Rhodotorula* species not only restricted the growth of the fungus but also reduce sporulation (Waff- Haggeg *et al.,* 2014). From this study, mixed growth of *Aspergillus flavus* + *Aspergillus fumigatus* and *Aspergillus flavus vs Aspergillus nidulans* have no noticeable effects on aflatoxin production.

In this study (Table 4.7), out of the total of 83 *Aspergillus flavus* isolates encountered from varoius cashew nuts, only 17 samples of cashew nuts were found to produce aflatoxins using the Rida® quick aflatoxin immunchromatographischer test kit. This is consistent with previous reports. According to Moss (1996), on a worldwide basis, only about 35% of strains of *Aspergillus flavus* produced aflatoxins. Magnolia *et al.* (1998) observed that only 47% of the strains encountered in Argentina produced aflatoxins. Pitt (1993) also showed in his studies that only a small proportion of *Aspergillus flavus* produce aflatoxins. The general finding in this study on toxin production stresses the importance of substrates as governing factors in secondary metabolites synthesis. According to Cegler (1975), there is high proportion of toxin producing strains of *Aspergillus flavus* isolated from nuts and cotton seeds than from rice and sorghum in his work.

The results obtained from the natural occurance of aflatoxins in cashew nut substantiate the probability of aflatoxins ingestion by Nigerians. The high incidence of naturally produced aflatoxins illustrates the hazards with which the country is confronted. The actual amount of aflatoxin levels obtained for most food stuffs when compared with the tolerance limit of 30ppb decided by the FAO (FAO 1997) leaves no doubt regarding the existence of aflatoxins contamination in the country. Many samples of cashew nuts which yielded *Aspergillus flavus* growth gave negative aflatoxin result. A lot of factors may account for this observation including environmental factors, the substrates and mix growth ecosystem. Findings in this study generally cashew nuts are good substrate for aflatoxin production. This is consistent with other observations (Moss, 1989; Mishra and Daradliyar, 1991; Russel *et al*., 1991; El-kady *et al.,* 1994; Julian *et al.,* 1995).

Table 4.8 shows the weight of the wistar rats after 10 days of feeding.It indicated that intoxication with aflatoxins resulted in a significant reduction in body weight. Similar decrease in body weight was reported in rats fed with AFB_1 ï contaminated diet Madrigal ï Santillan *et al.* (2006, 2007). The reduction in body weight may be explained by the ability of Aflatoxins to generate free radicals Abdel ï Wahhab *et al.* (2008) which may lead to DNA breakage, inhibition of protein synthesis and gluconeogenesis lipid, peroxidiatIon, disruption of oxidative phosphorylation in mitochondria, inhibition of blood clotting and apoptosis Paarhizkar *et al.* (2002). Furthermore, the decrease in body weight in animals treated with AFB1 may be due to their effect on the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation leading to cachexia (Rastog *et al.,* 2007). Abdel-Wahhab *et al*. (2006) reported that rats treated with AFB_1 showed a decrease in leptin which act together to regulate energy balance and body weight control. These influence the feeding response causing weight loss Yuan *et al*. (2004). This disagrees with the current result of Onifade *et al.* (1999) which says that Sc gavage before Aflatoxin administration enhanced growth and resulted in a significant recovery in body weight. This study also contradicted the work of De Groofe (1992) and Madrigal ï Sbntillan *et al.* (2006) which indicated that animals treated with the Sc plus Aflatoxin, gained considerable weight in the 3rd and 6th weeks of the assay which was more than double in the usual level reached by aflatoxin treated mice. Increase in weight has been previously observed in adult and juvenile fish and sea bass larvae fed with liver yeast Lara- Flores *et al.* (2003).

Table 4.9 herein showed biochemical changes in liver and kidney function tests and calcium level. The activity of ALT and AST is a sensitive indicator of acute hepatic Necrosis and hepato-biliary disease. Increase in AST and ALT activity indicates initial hepato-celluar damage. This

agrees with the work done by Abdel- Wahhab *et al.* (2006). Treatment with aflatoxin was found to alter serum biochemical parameters. In this study, there was slight increase in serum ALT and AST in two wistar rats. AST and AST activities were observed as a result of effect of aflatoxin contaminated cashew nut. Similar to our results, Aravind *et al.* (2003) reported increase as a result of aflatoxin B_1 treatment. Aravind *et al.* (2003) reported increase in ALT and AST in broiler chickens fed aflatoxin contaminated diet. Preetha *et al.* (2006) and Yener *et al.* (2009) also reported that aflatoxin contaminated diet significantly increases the activities of all serum ALT and AST. Such increase can be attributed to cell necrosis, changes in cell membrane, permeability of impairment of biliary excretion (Pozzi *et al*., 2010).

The finding also agrees with previous studies that reported the elevation of creatinine, in serum of rabbits receiving aflatoxin contaminated feed (Verma and Raval, 1997; Ghaly *et al.,* 2010). It is well established that any changes of creatinine and urea levels in serum is suggesting impairment of kidney functions.

Plate 4.5 shows the light photomicrograph of the liver section of the control rat showing normal histomorphology. The central vein, hepatocytes, sinusoids and portal tract are normal (Stain H & E/ Mgig: x 400).

Plate 4.6 shows light photomicrograph of liver section of the test rat fed with 50% cashew nut diet showing marked widening of the sinusoids, central canal rupture, degrading hepatocytes and inflammatory cellular infiltration especially within the sinusoids (Stain H &E/Mag:- x 400) suggesting liver cirrhosis.

This agrees with the work done by (Darnish *et al.,* 2011) whose work recorded histological evaluation aflatoxin intoxicated liver showing distortion of hepatic architecture with dilated and congested central vein. Ballooning of cytoplasm and darkening of nuclei were also noticed.

Plate 4.11 shows the light photomicrograph of kidney sections of control rats showing normal structures

Plate 4.12 shows the light photomicrograph of kidney sections of rat fed with aflatoxin contaminated cashew nuts showing normal structures. This disagreed with the work done by Mollenhaure *et al*., (1989), Ezz El ï Arab *et al.* (2006) and Orsi *et al.* (2007) who recorded that histologic section of mice renal tissue treated with aflatoxin showed hyper-cellularity and severed degeneration in glomeruli and tubules. The obtained data are in agreement with the previous literature, where aflatoxin administration which induced cloudy swelling of the epithelial lining of renal tubules with mild intestinal fibrosis and congestion and frequent vacuolar degeneration.

Data presented in Table 4.9 herein showed no significant changes in electrolytes test (acid-base balance) of the test and control albino wistar rats. There was no significant difference between the two groups $P > 0.05$). Present findings also indicated that AFB_1 treatment induced significant increase ($P < 0.05$) in calcium level of the test group serum when compared with the normal control group.

CONCLUSION

The results of this study indicate that cashew nuts are some of the best substrates to harbor *Aspergillus flavus* which has the ability to produce aflatoxins causing liver cancer and other fungal infections. In Enugu state, Nigeria, cashew nuts are largely consumed as a common snack thereby posing a risk to itôs consumers. Animal studies revealed some inflammatory lesions in the liver suggesting cirrhosis.

Animal studies also revealed changes in renal functions. Packaging of cashew nuts in hand knotted- polythylene bag is a major factor that increases *Aspergillus flavus* contamination due to high temperature and humidity.

Interactions of *Aspergillus flavus* with other moulds dramatically influenced aflatoxin production and showed inhibitory effects.

RECOMMENDATION

The result of the study suggests that the consumption of contaminated cashew nuts which is a major snack food widely patronized in Enugu, may pose a serious public health hazard that may target the liver aside other minor issues. This therefore emphasizes the pertinent need for cashew nut protection studies and implementation in line with the recommendation of the FAO (Food and Agricultural Organization) and WHO (World Health Organization).

Post-harvest processing, packaging and marketing have been commercialized and modern technology and regulations adopted in countries like Brazil, India, Mexico and Tanzania.

REFERENCES

Abdel ï Wahhab M. and Aly, S. (2000). Antioxidants and radical scavenging properties of vegetable extracts in rages fed aflatoxin contaminated diet. *Journal of Agric and Food Chem.,* 51: 2409 ï 2414.

Abdel ï Wahhab, M., Ahmed, H. and Hajazi, M. (2008). Prevention of aflatoxin B_1 initiated hapatoxicity in rat by marine alga extracts. *J. Appl. Toxicol*., 26 (3): 229 ï 238.

Adebajo, L.O. and Idowu, A.A. (1994). Mycoflora and aflatoxins in a West Africac corn-groundnut based convenience food. *Mycopathologia* 126(1): 21-26

Agarwala, C.P. (1986) Chatterjee, C., Nautiyal N. and Sharma C.P. (1986). Molybdenum nutrition of isolates of four *Aspergillus species*.*Canadian Journal of Microbiology*. 32(7): 557-561.

Agboola, S.D. (1990). Postï harvest technologies to reduce mycotoxin contamination of food crops. Proceedings of the National Workshop on Mycotoxins, Jos, 28th -30th Nov. 74 -83.

Adebejo, L.O. and Diyaolu, S.A. (2003) Mycology and Spoilage of retail Cashew Nuts. *African Journal of Biotechnology* (10): 369 -373.

Alawodi, S.E., Atitu, A.A. and Lamorde, A.G. (1994). Aflatoxin contamination of Nigerian foods and feeding stuffs. *Food Chem and Toxicology* 32(1):61-63.

Ali, N., Sardjone, T., Yamashita, A. and Yoshizawa, T. (1998). National- co-occurrence of Afltoxins and Fusarium mycotoxins (fumonisins, deoxyniyalenol, nivalenol and zearalenone) in corn from Indonseia, *Food Additives and Contaminant* 15(4): 377 ï 384.

Amuta, E., Houmsou, R. and Mker, S. (2006) Impact of socio-demogrpahical and economic factors on the prevalence of intestinal parasites among the female gender in Makurdi Benue State Nigeria. *The Internet Journal of Third World Medicine* 8(2).

Arseculeratne, S.N. and Bandunatha, C.H.S.R.(1972). Variation of Aflatoxin content of cultures of Aspergilliusflavus with duration of incubation and its relation to studies on aflatoxin production. *Journal of Applied Bacteriology.* 35 :43-52.

Avarind, K. L., Patil, V.S., Deregowda, H., Umakanthat, B. and Guanpule, S.P. (2003). Efficacy of esterifed glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum Biochemical and hematological parameters in broilers. Poultry Sci. 82: 571- 576.

Bavier, J. (29 October 2014). ñWar-scarred Ivory Coast aims to conquer the world of cashewsò. Reuters. Retrieved 9 February 2015.

Bacha, H. Hadidane, R. Creppy, E.E. Regnault, C. Ellouze, F. Dirheimer, G. (1988) monitoring and identification of fungi toxins in food products animal feed and cereals in Tunisha *J. Stored Prod. Res*. 24: 199 -206.

Betina, V.C. (1998), *Mycotoxins, Chemical, Biological and Environmental aspects*. Elsevier, Amsterdam.

Bhat, R.V. (1996). Aflatoxin and liver injury. *Tropical Gastroenterology* Jul ï Sept; 17(3): 153-160.

Blomhoff, R., Carisen, M.H., Andersen, L.F. and Jacobs, D.R. Jr. (2006). Health benefits of nuts: potential role of antioxidants. *Br J Nutr*. 96 Suppl 2:S52-60. 2006. PMID: 17125534.

Blout, W.P. (1961). Turkey ñXò disease. *Turkeys* 9:52, 55- 58.

Caju, identidade tropical que exala saüde — Embrapa". Embrapa.br. Retrieved 2012-12-22.

Caju, identidade tropical que exala saude ï embropaò. Embrapa. br. Retrieved 2012-12-22.

Cajucultura historia (in Portuguese)ò Retrieved February 2, 2010.

Cajucultura historia ñ(in Portuguese)ò. Retrieved February 2, 2010.

Chelkowski, J. (ed) (1991). *Cereal Grains Mycotoxins, Fungi and Quality in Drying and Storage*. Elservier; Amnsterdam.

Coulomb, R.A. Jr. (1993). Biological action of mycotoxins.*Journal of Diary Sciences* 76(3): 880-891.

Cobley, L.S. and Steele, M. (1976). *An introduction to the Botany of tropical crops* 2nd ed. Longman Group Ltd., London

Crowther, P.C. (1973). Report to the Government of Nigerian on aflatoxin in groundnut and groundnut products. UNDP project. *Food and Agriculture Organization (FAO) REPORT NOTE*. 3221, pp 33-36.

Cullen, J.M. and Newberne, P.N. (1994). Acute hepatotoxicity of aflatoxins. In Eaton D.L. and GroopmanJ.J. (Ed.). *The Toxicity of Aflatoxins: Human Health, Veterinary and Agricultural Significance*. Academic Press, San Diego (USA)., 3 ï 26.

Cultivating Cashew Nuts (2015) ARC-Institute for Tropical and Subtropical Crops, South Africa. Retrieved February 15, 2015.

Darnish, H. R., Omara, E.A., Abdel- Aziz, K.B., Faragi, I.M., Nada, S.A. and Tawfek, N.S. (2011). Saccharomyces cerevisiae modulates aflatoxin- induced toxicity in male albino Mice. *Report and Opinion* 3 (12): 32 ï 43 (ISSN: 553 ï 9873).

Davis, C.D. (2003). Low dietary copper increases fecal free radical production, fecal water alkaline phosphatase activity and cytotoxicity in healthy men. *J Nutr.* 133(2):522-7. 2003.

Dhir, V. and Mohandas, K.M (1998). Epidemiology of digestive tract cancers in India. III.Liver. *Indian Journal of Gastroenterology* 17(3): 100 ï 103.

Diener, U. L., Cole, R. j., Sanders, T.H., Hayne, G.A., Lee, L.S.and Klich, M.A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus, Annu. Rev. Phytopathol*. 25, 249-270.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee L.J. and Keich, M.A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus. Annual Review of Phytopothology* 25:249-270.

Diprossimo, V.P. and Malek, E.G. (1996). Comparison of three methods for determining aflatoxins in melon seeds. *Journal of the Association of Official Analytical Chemists International.*79(6): 1330-1335.

Eaton, D.L.and Gallagher, E.P. (1994). Mechanism of aflatoxins carcinogenesis. *Annu. Rev. Pharmacol.Toxicol*., 34, 135 ï 172.

Eaton, D.L., Groopman, J.D.and Ed., (1994). *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. Academic Press, San Diego (USA).

Egmond, H.P. and Van, P. (1989).Current situation on regulation for mycotoxins. Overview of tolerance and status of standard methods of sampling and analysis. *Food Additives and Contaminants* 6: 139 ï 188.

El- Agamy, D. (2010). Comparative events of Curcumin and resveratrol on aflatoxin B1 ï induced liver injury in rats. *Arch. Toxicol*., 84 (5): 389 ï 96.

Ellis, W.O., Smith, J.P., Simpson, B.K. and Oldham, J.H. (1991). Aflatoxin in food: occurrences, biosynthesis, effects on organism detection, and methods of control.*Critical Reviews in Food Science and Nutrition.*30(4):*403 -439.*

Ensminger, A.H., Ensminger, M.E., Kondale, J.E., Robson, J.R.K. *Foods and Nutriton Encyclopedia*. Pegus Press, Clovis, California. 1983.

Ensminger, A.H., Esminger, M. K. J. (1986). *Food for Health: A Nutrition Encyclopedia*. Clovis, California: Pegus Press; 1986. 1986. PMID:15210.

Esurusoso, O.F.(1974) Fungi associated with kernel rot disease of cashew (Anacardium occidentale L.) in Nigeria. *Int. Biodetn. Bull*. 10: 57-59.

Ezz El- Arab, A., Girgis, S., Hegazy, E. and Abd El- Khalek, A. (2006). Effect of dietary honey on intestinal microflora and toxicity of Mycotoxins in mice BMC *Complementary and Alternative Medicine*, 6: 1 ï 13.

Fortin, Francois, Editorial Director. *The Visual Foods Encyclopedia*. Macmillan, New York. 1996.

Frazier, W.C. and Westerhoff. D.C. (1991): *Food Microbiology* 5th ed. Butterworth, London. Pp 84 ï 139.

Ghaly, I., Hassanane, M., Ahmed, E., Haggag W., Nada, S. and Farag, I. (2010). Cytogenetic and Biochemical studies on the protective and Role of Rhodotorula glutinis and its autophidy against the toxic effect of aflatoxin B_1 in Mice. *Nature and Science*, 8 (5) 28 ï 38.

Glossary, C-G. www.joyofbaking.com. iFood Media LLC. Archivedfrom the original on 2014-03-2 7.

Goto, T., Wicklow, D.T. and Ito, Y.(1996). Aflatoxins and cyclopiazonic acid production by a sclerotium-producing Aspergillus tamari strain. *Appl. Environ. Microbiol*., 62, 4036-4038.

Groopman, J.D., Donahue, K., Zhu J., Chen J. and Wogan, G.N. (1985). Aflatoxin metabolism in humans: Detection of metabolities and nucleic adducts in urine by affinity chromatography. *Proc. Natl. Acad. Sci USA* 82, 6492-6496.

McGraw, H. (2004). On food and cooking (see Nuts and other Oil ï rich seeds chapter) Scribner. ISBN 978-0-684-80001-1.

Heathcote, J.G. and Hibbert, J.R. (1978). Aflatoxins: Chemical and Biological Aspects. Elsevier Scientific Publishing Company, Amsterdam, The Netherlands.

Hsieh, D. (1988). Potential human health hazards of mycotoxins, In: Natori, S., Hashimoto K. and Ueno, Y. (Ed.), *Mycotoxins and Phytotoxins*. Third jont food and Agriculture Organization W.H.O. United Nations program International Conference of Mycotoxins. Elservier, Amsterdam, The Netherlands 69 ï 80.

Hu, F.B.and Stampfer, (1999). M.J. Nut consumption and risk of coronary heart disease: A review of epidemiologic evidence. *Curr Atheroscler* Rep 1999 Nov;1(3):204-9. 1999.

Ibeh, I.N., Uraih, N. and Ogonor, J.K.I. (1991): Dietary exposure to aflatoxin in Benin-city, Nigeria: a possible Public Health concern.*International Journal of Food Microbiology 14(2): 171 -174.*

International Agency for Research on Cancer (1982). The evaluation of the carcinogenic risk of chemicals of humans. JARC Monograph Supplement 4 International Agency for Research on Cancer, Lyon France.

Jones, F.T., Hsgler, W.H. and Hamilton, P.B. (1984). Correlation of aflatoxin contamination with zinc content of chicken feed. *Applied and Environmental Microbiology* 47: 478 ï 480.

Kelly, J.H. Jr. and Sabate, J. (2006). Nuts and coronary heart disease: an epidemiological perspective. *Br. J. Nutr*. 96 Suppl 2:S61-7. 2006. PMID:17125535.

Kurata, H. and Ueno, Y. (1984). *Toxigenic fungi- Their Toxins and Health Hazard*. Amsterdam: Elsevier.

Lacey, J. (1989): Pre and Post harvest ecology of fungi causing spoilage of foods and other stored products. *Journal of Applied Bacteriology Symposium Supplement.*IIS-25S.

Lacey, J. and Crook, B. (1988). Fungal and Actinomycetes spores as pollutants of the Workplace and Occupational Allergens ï Annual Occupational Hygiene. 32: 515 -533.

Lamble, L. (2013). Cashew nut workers suffer óappallingô conditions as global slump dents profitsò. The Guardian Retrieved 6 September 2015.

Lara-Flores, M., Olvera- Novoa, M., Guzman- Mendez, B.and Lope z ï Madrid, W. (2003). Use of the bacterial *Streptococcus feacium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces* in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216 : 193- 201.

Lugaskas, A., Mikulskiene, A. and Sliauziene, D. (1987). Catalogue of Fungi deteriorating Polymeric Materials-Nauka, Moscow (in Russian).

Madrigal- Santillan, E., Madrigal- Bujaider, E., Marquez ï Margues, R. and Reyes, A. (2006). Anti genotoxic effect of saccharmyces cerevisiae on the damage produced in mice fed with aflatoxin B_1 contaminated corn. *Food and Chemical Toxicology*, 44 2058 ï 2063.

Maertens, L. and De Groote, G. (1992). Effect of a dietary supplementation of liver yeast on the 200 technical performances of dose and weaning rabbits. *J. Appl. Rabbit Res.*, 15: 1079 ï 1086.

Major Food and Agricultural Commodities and Producers Countries By Commodity *(FAO)*. 2013. Retrieved 6 September 2015.

Martinez-Gonzalez, M.A. (2007). Nut consumption and weight gain in a Mediterranean cohort: The SUN study. Obesity (Silver Spring). 15(1):107-116. 2007.PMID: 17228038.

McDonald, S. and Castle, L. (1996). A Uk retail survey of aflatoxins in herbs and species and their fate during cooking.*Food Additive and Contaminants.* 13(1): 121 ï 128.

Mollenhaver, H., Corrier, D., Huft, W., Kybena, L., Harery, R. and Droleskey, R. (1989). Ultrastructure of hepatic and renal lesions in chickens fed aflatoxin. *Am. J. Vet. Res*., 50: 771 ï 777.

Moss, M.O. (1989). Mycotoxins of filamentous fungi. *Journal of Applied Bacteriology Symposium Supplement.* 69S ï 79S.

Moss, M.O. (1996). Centenary Review Mycotoxins *Mycology Resaerch* 100(5): 513 ï 523.

Moss, M.O. (1989) Recent studies of mycotoxins *J. Appl. Microbiol* symposium Suppl: 84,62S-76S.

Nidaâ Shihab Hamed, Abeer Fauzi Murad and Eman Abdul ï Wahed Abdula ï Rahim (2016). Molecularly Diagnositic of aflatoxigenic *Aspergillus flavus* isolated from nuts. *Research Journal of Environmental Toxicology,* 10:39-49.

Oõ Neil, M.J., Smith, A. and Heckelman, P.E. (2001). *The Merck Index,* 13th Ed., White house Station, NJ, Merck & Co., (USA), 34- 35.

Obidoa, O. and Onyeneke, E.C. (1980). Effect of sucrose supplementation on aflatoxin production by *Aspergillus parasiticus* 2999 culutres in soybeans and cashew fruit juice. *Mycopathologia.* 70(1): 33 -36.

Obidoa, O. and Gugnani, H.C. (1990). Mycotoxins in Nigerian Food: Causes, consequences and remedial measures. Proceedings of the National Workshop on Mycotoxins, Jos, 28th ï 30th Nov. 95 ï 108.

Onifade, A., Obiyan, R., Onipede, E., Adejumo, D., Abu,O. and Babatunde, G. (1999). Assessment of the effect of supplementing rabbit diets with a culture of Saccharomyces cerevisiae using growth performance blood composition and clinical enzyme activities. *Anim. Feed Sci. Technol*., 77: 25 ï 32.

Opadokun, J.S. (1979): The aflatoxin contents of locally consumed foodstuffs. Part 1: Gari: *Annual Report of Nigerian Stored Products Researcher Institute* (1975/76), 45 ï 50.

Opadokun, J.S. (1979): The aflatoxin contents of locally consumed foodstuffs. Part II: sorghum i id. (1976/77),101 -104.

Palermo, Elizabeth (June 30, 2014). Where Do Cashews Come From? Live science.

Park, D.L. (1995). Surveillance programmes for managing risks from naturally occurring toxicants.*Food Additive and Contaminants.* May-Jun; 12(3): 361 -371.

Phillippa Cheifitz (2009). South Africa Eats.

Pitt, J.I. (1993). Corrections to species names in physiological studies on *Aspergillus flavus* and *Aspergilusparasiticus. Journal of Food Protection.*56:265-269.

Pitt, J.I.and Hocking, A.D.(1985). *Fungi and Food spoilage*, Academic Press, London, pp. 339-416.

Pitt, J.I. and Miscamble, B.F. (1995). Water relations of *Aspergillus flavus* and closely related species. *Journal of Food Protection*. 58: 86 ï 90.

Raper, K.B. and Fennel, D.I. (1965). *The Genues Aspergillus.* The Williams and Wilkins Company, Baltimore.

Resnicow, K., Barone, J., Engle, A. (1991). Diet and serum lipids in vegan vegetarians: a model for risk reduction. *J Am Diet Assoc*. 91(4):447-53. 1991. PMID:1610.

Resnik, S., Neire, S., Pacin A., Martinez, E., Apro, N. and Laterite, S. (1996). A survey of the natural occurrence of aflatoxins and zearalenone in Argentine field maize: 1983 ï 1994.*Food Addiitive and Contaminats.* Jan. 13(1): 115 -120.

Rittera, M. (2007). Soluble and insoluble oxalate content of nuts. *Journal of Food Compositon and Analysis* 20 (3-4): 169 ï 174 doi:10.1016/ j.jfca.2006.12.001.

Rosen, T. and Fordice, D. B. (April 1994). ñCashew Nut Dermatitisò.*Southern Medical Journal* 87 (4): 543ð 546. Doi;10.1097/00007611-199404000-00026. PMID 8153 790. Retrieved 2011-01-13.

Smith, J.E. and Moss, M.O. (1985). *Mycotoxins, Formation, Analysis and Significance.* John Wiley: Chichester.

Smith, J.E. and Henderson, R.S. (ed.) (1999). *Mycotoxins and Animals Foods*. CRC Press: Florida.

Squire, R.A. (1981). Ranking animal carcinogens: *A proposed Regulatory Approach. Science.* 214: 877-880.

Strzelecki, E.I., Gasiorowska, U., Gorazdowska, M., Cader-Strzelecka, B. and Pawelezak, M. (1988). Levels of aflatoxin $B_{1,}$ bacteria and fungi in feed and food 1971 ï 1987. *Mycotoxin Research*, 4:2, 89 ï 96.

Tanzania riots over cashew nut payments. BBC. 24 April 2013. Retrieved 14 May 2013.

Tsai, C.J., Leitzmann, M.F., Hu, F.B., Willett, W.C., Giovannucci, E.L. (2004). Frequent nut consumption and decreased risk of cholecystectomy in women. *Am J Clin Nutr*. 80(1):76-81. 2004. PMID:15213031.

Van Egmond, H.P. (1995). Mycotoxins: regulations, quality assurance and reference materials. *Food Additives and Contaminants*. May-Jun; 12(3): 321 ï 330.

Varghese, T.and Pundir, Y. (1964). Anatomy of the pseudocarp in *Anacardium Occidentale L.Proceedings: Plant Sciences.* 59(5): 252-258.

Verma, R. and Rava,l P. (1997). Nephrotoxicity during aflatoxicosis. *Med. Science Res*., 25: 655 ï 657.

Weckbach, L. S. and Marth, B.F. (1977). Aflatoxin production by *Aspergillus parasitius* in a competitive environment. *Mycopathologia.* 62(1): 39 ï 45.

Wilson, B. (4 May 2015). Blood cashew: The toxic truth about your favourite nuts. The Telegraph. Retrieved 6 September, 2015.

Wilson, D. M. and Payne, G.A. (1994).Factors affecting Aspergillus flavus group infection and aflatoxin contamination of crops, in Eaton D.L. and Groopman, J.D. (Ed.). *The Toxicology of Aflatoxins. Human Health, Veterinary and Agricultural Significance.* Academic Press. San Diego (USA). , 309-325.

WHO, (2006). Impacts of aflatoxins on health and nutrition, http://services.leatherheadfood.com/ema/factshet.aspx

Wood, Rebecca. *The Whole Foods Encyclopedia*. New York, NY: Prentice-Hall.

Yener, Z., Celik I., Rhan, F. and Bal, R. (2009). Effects of urtica dioica L. seed on lipid peroxidation, antioxidants and liver pathology in aflatoxin- induced tissue injury in rats. *Food Chem. Toxicol*., 47: 418 ï 424.

Yoshizawa, T., Yamashita, A. and Lou, Y. (1994). Fumonisin occurrence in corn from high and low risk areas for human oesophageal cancer in China.*Applied and Environmental Microbiology.*60: 1626 ï 1629.

Yuan, S., Tsai, K.J., Chung, Y., Uhan, Tsat L. and Su, J. (2004). Aberrant expression and possible involvement of the Leptin Receptor in endometrial cancer. *Gynecol. Oncol*., 92: 769 ï 775.

Zhu, C. R., Du, M. J., Lei, D. N and Wan, L.O. (1989). A study on the inhibition of aflatoxin B_1 induced hepatocarcinogenesis by the *Rhizopus delemar*. *Mater-med-poi*: Apr. ï Jun; 21 (2) 87 ï 91.

Zohri, A.A. and Saber, S.M. (1993). Flamentous fungi and mycotoxin detected in coconut.*Zentrale-Mikrobiol.* 148(5): 325 ï 332.

APPENDIX

Biochemical test results for the laboratory animal (wistar rats)

Electrolytes result

Sample (1)	Na+	K^+	Cl^-	Ca	pH	nCa	Temperature
B1	140	7.1	111	o.81	high	1.18	37.0^0c
B_2	147	7.3	116	0.90	7.79	1.12	37^0c
B_3	142	7.5	110	0.96	7.85	1.24	37^0c
B_4	140	6.6	113	0.90	7.87	1.18	37^0c

Liver function test

Sample (1)	ALP	ALT	AST	CRE	HCO3	BILD	UREA	$BILT_3$
B_1	453.2	70.6	203.10	0.6	10.6	0.0	35.06	0.0
B_2	1178.2	132.2	161.8	0.6	7.6	0.0	32.46	0.0
B_3	629.5	116.0	157.6	0.4	14.4	0.0	22.48	-0.1
B_4	316.1	51.1	116.2	0.4	6.0	0.0	33.43	0.0

Key

ALP - Alkaline phosphatase

ALT - Alanine Transaminase

AST - Aspartate Transaminase

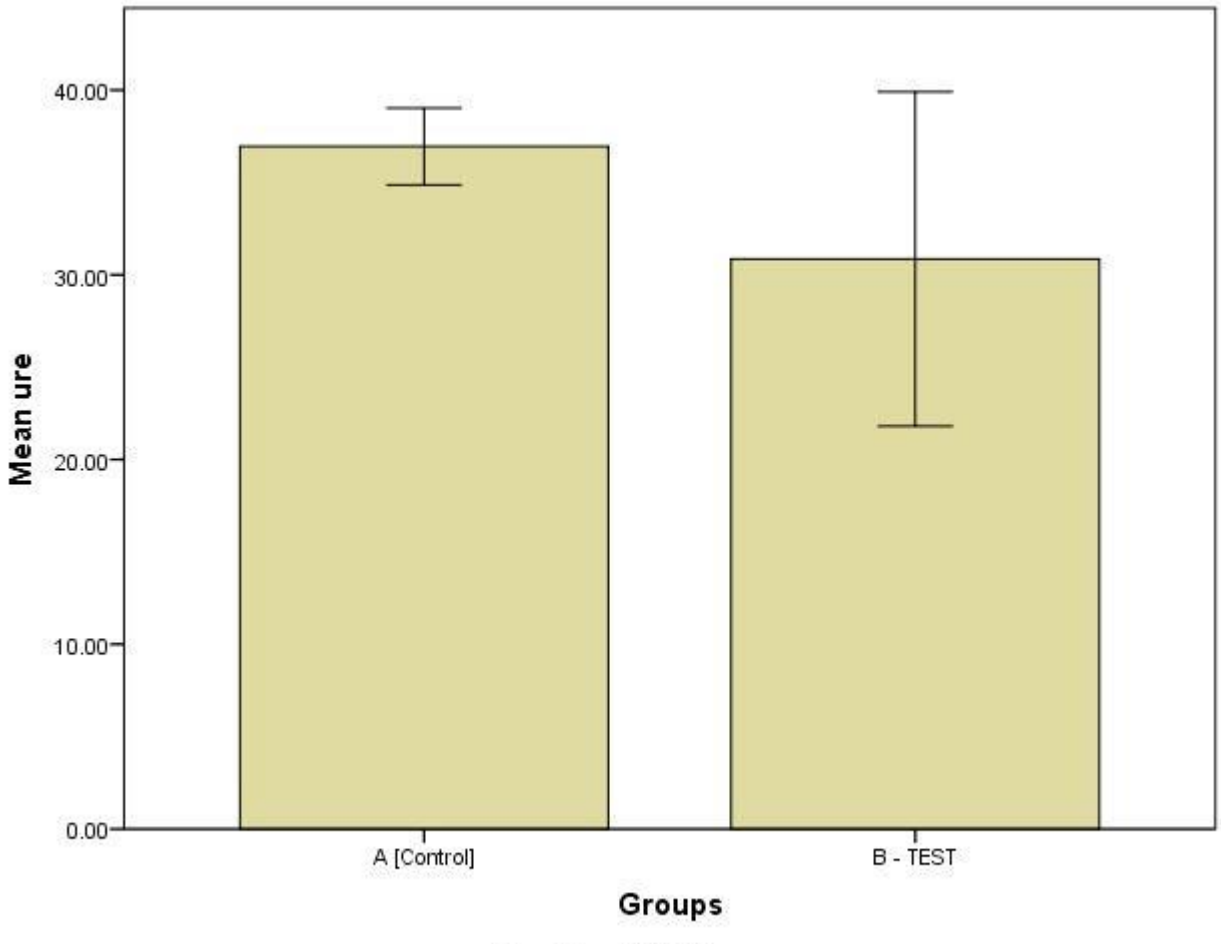
CRE - Creatinine

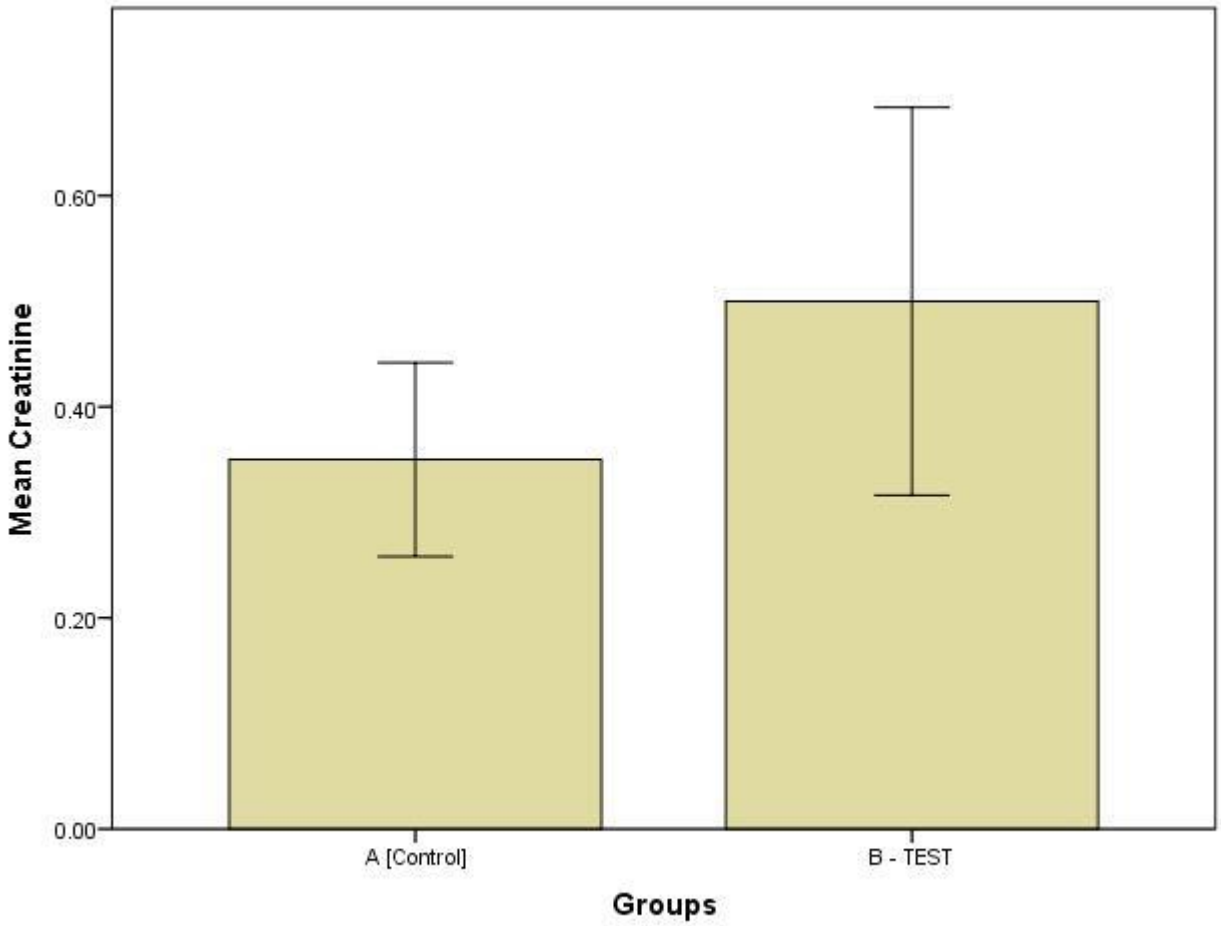
HCO3- Bicarbonate

BILD-2 Direct bihirubin

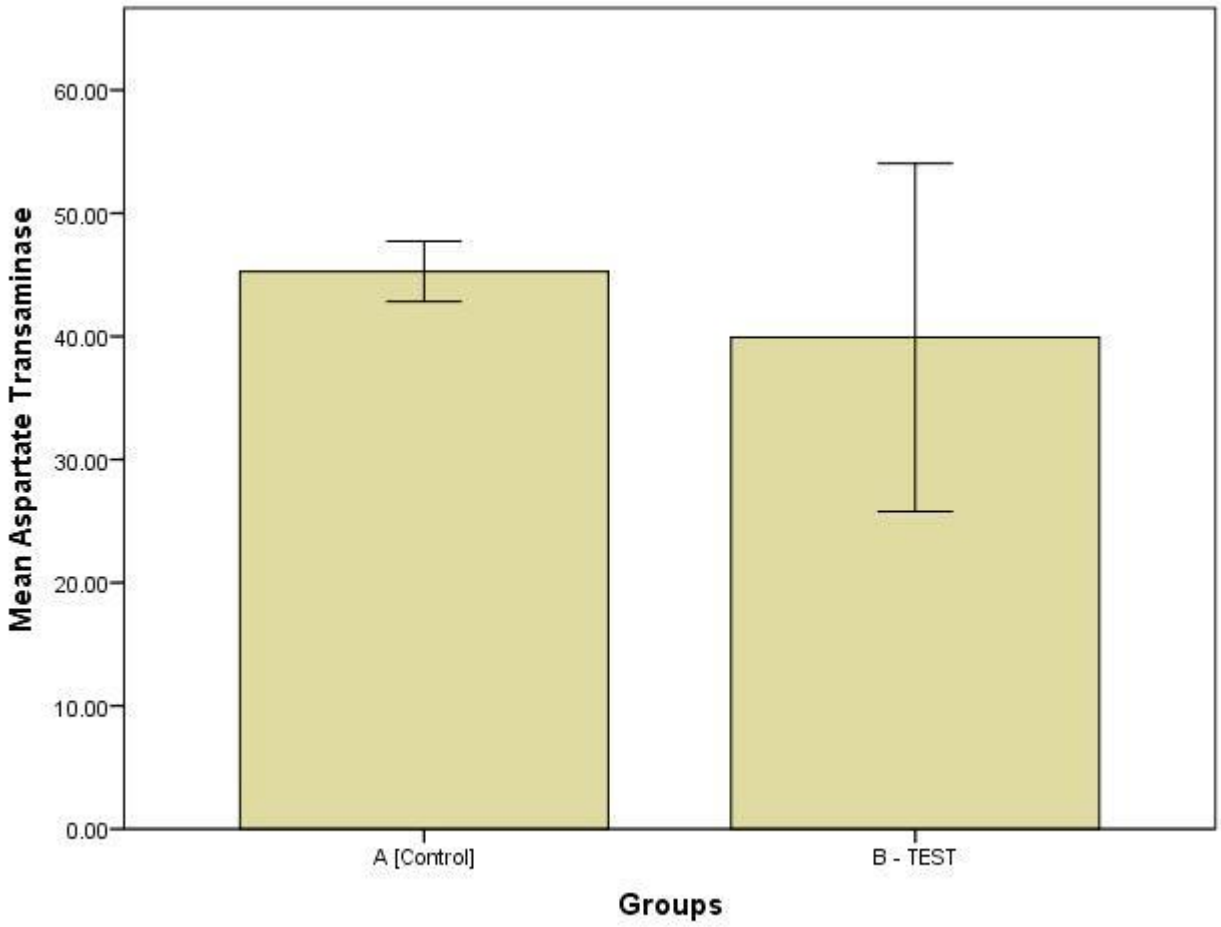
Urea - Urea

$BILT_3$ - Total bihirubin

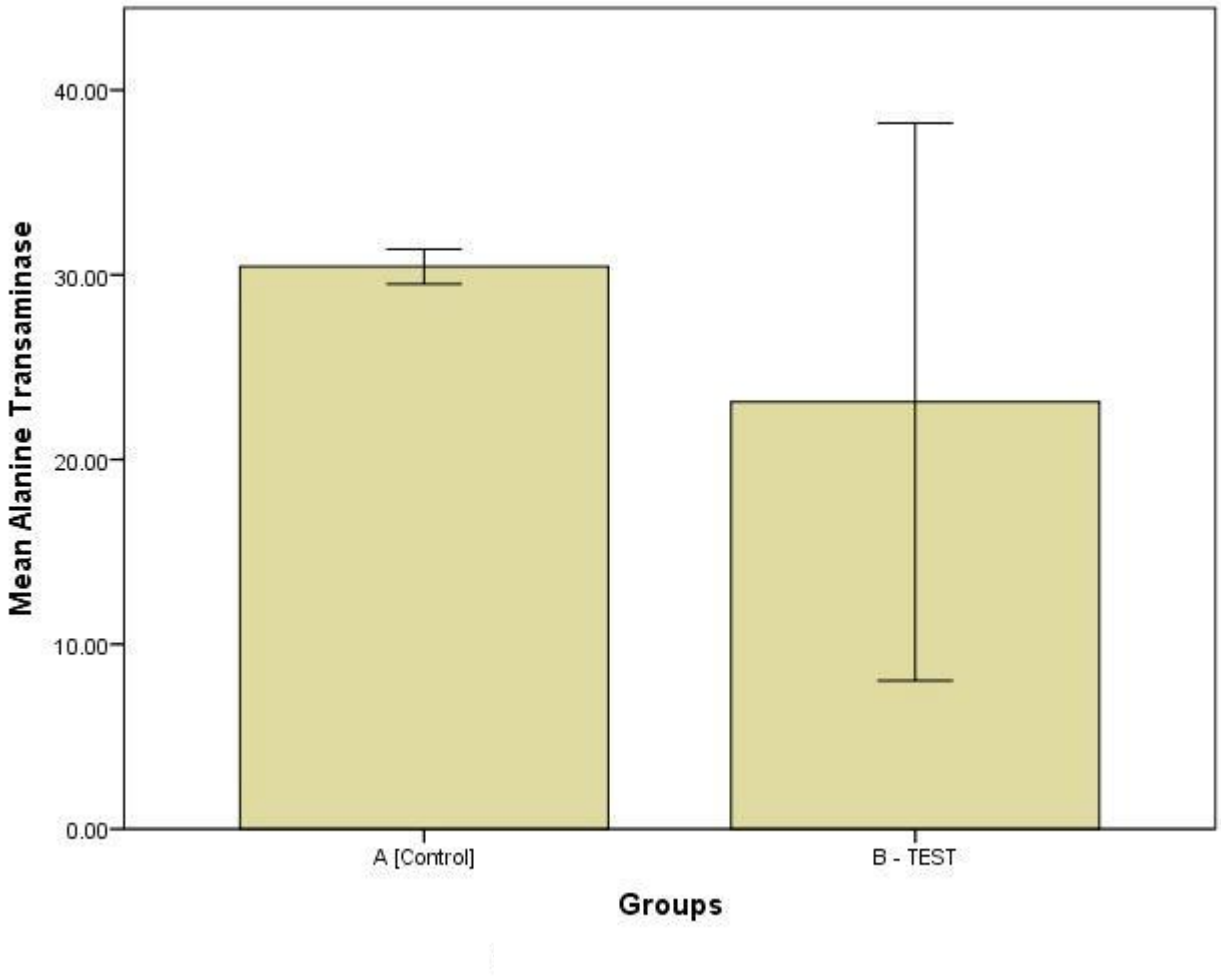

40.00
30.00
20.00
10.00
0.00
Mean ure
A [Control]
B - TEST
Groups



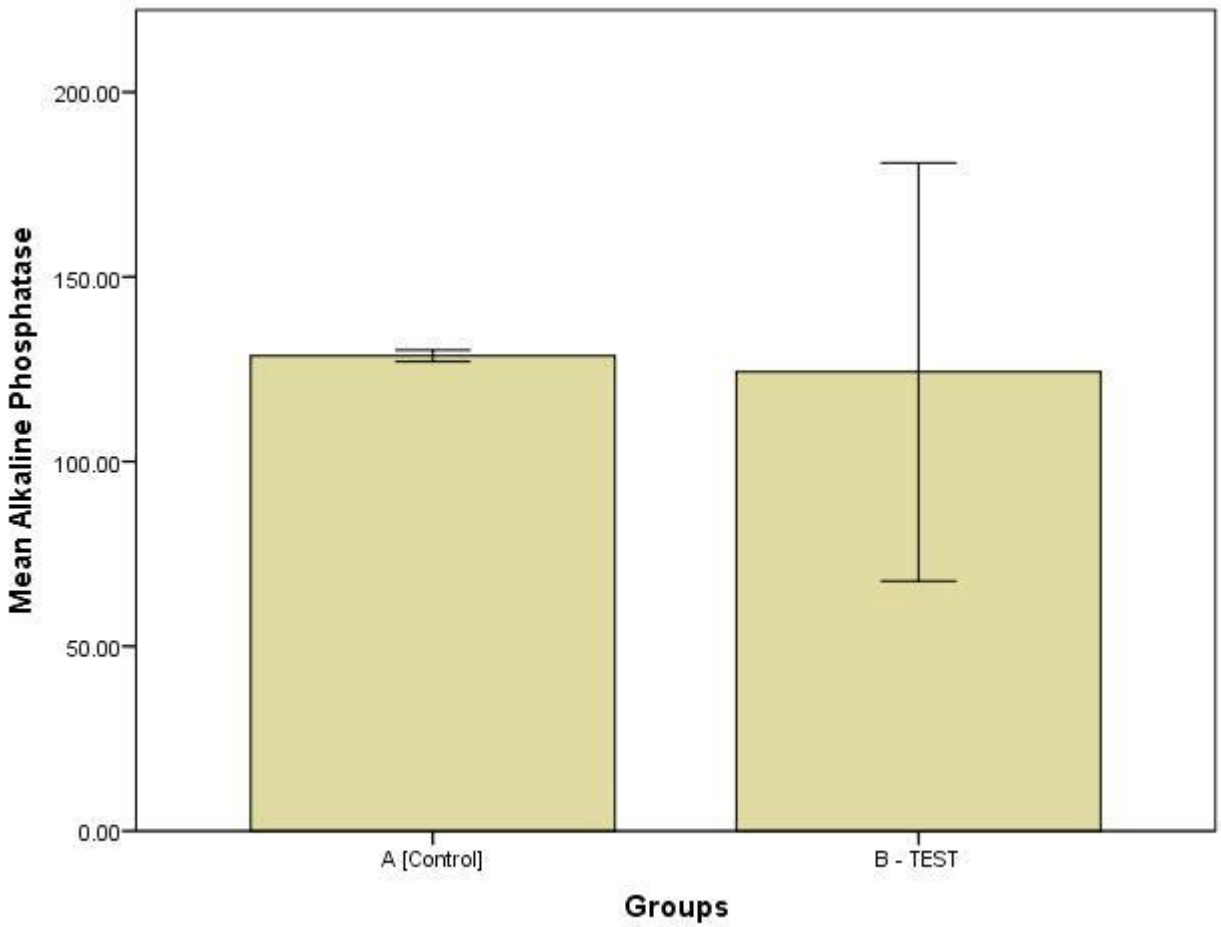
Creatinine mean value of wistar rat. Appendix 1a



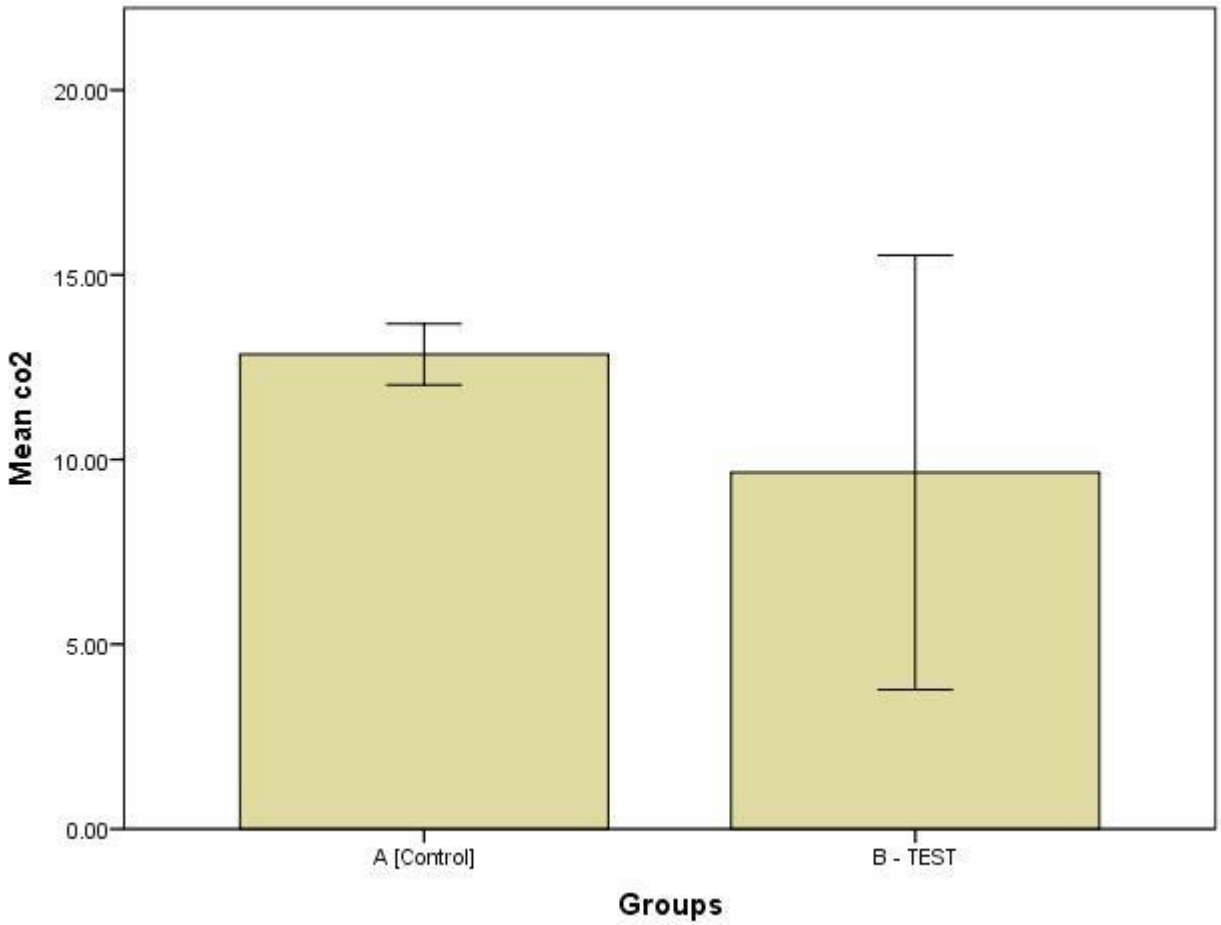
Aspartate transaminase mean value wistar rat. Appendix 1b



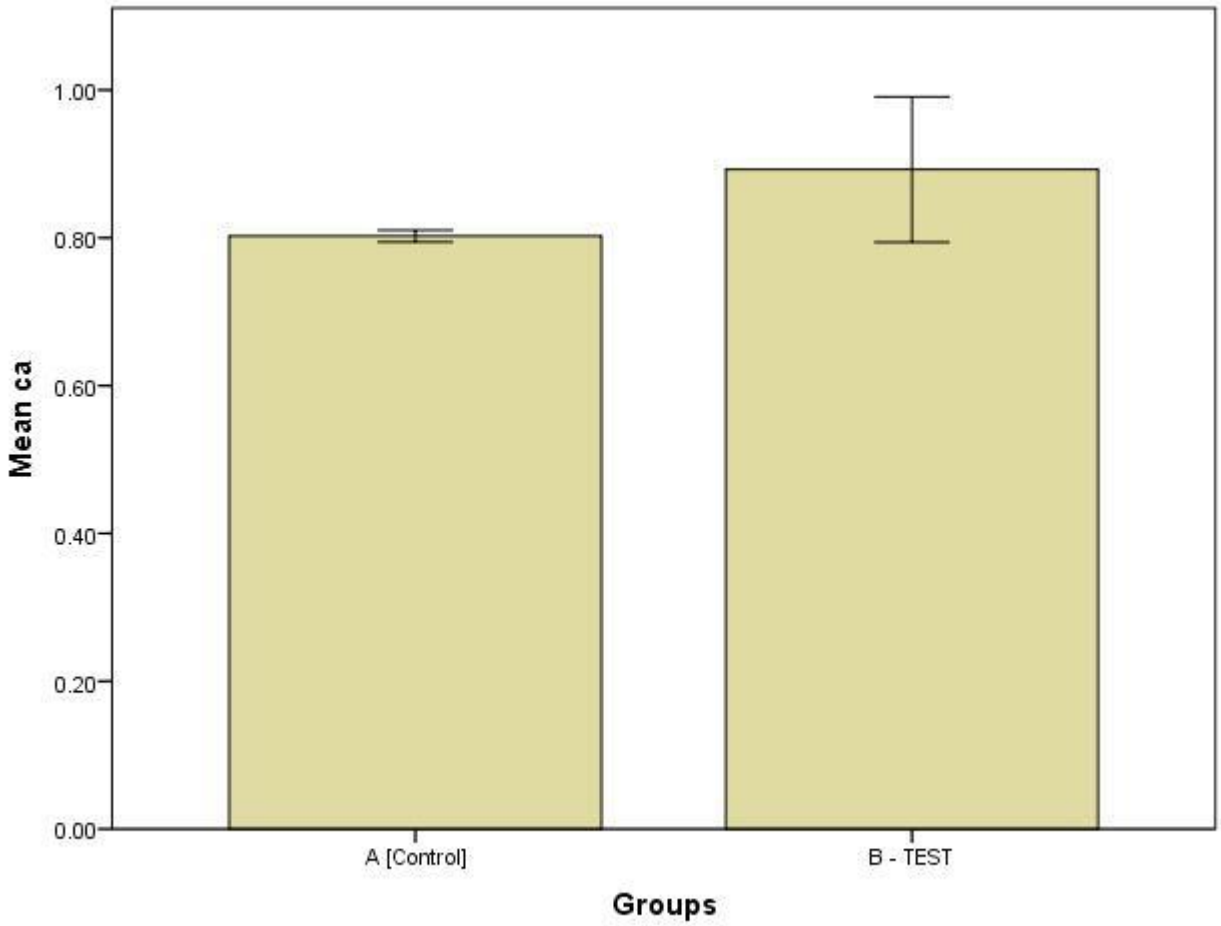
Alanine transaminase mean value wistar rat. Appendix 1c



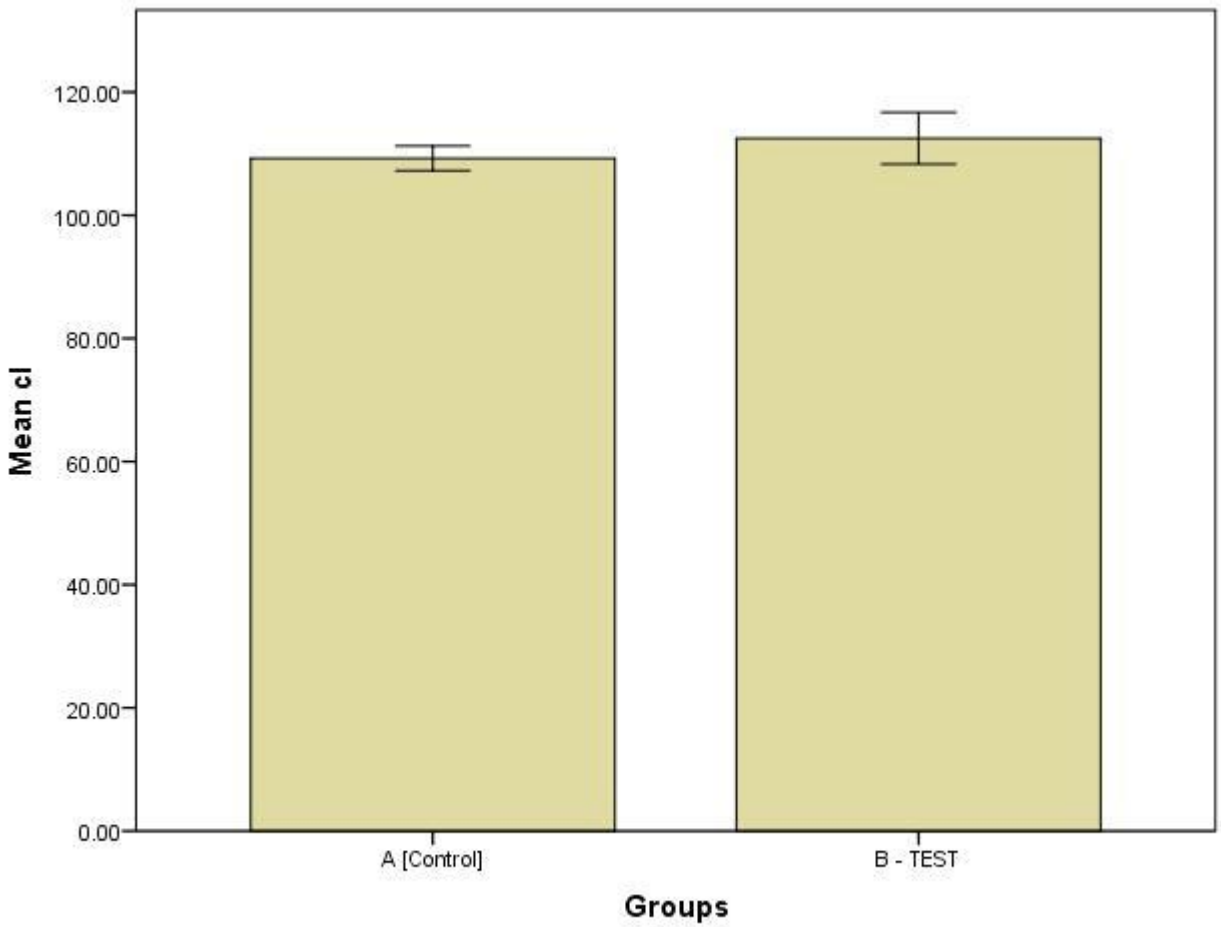
Alkaline phosphatase mean value of wistar rat. Appendix 1d



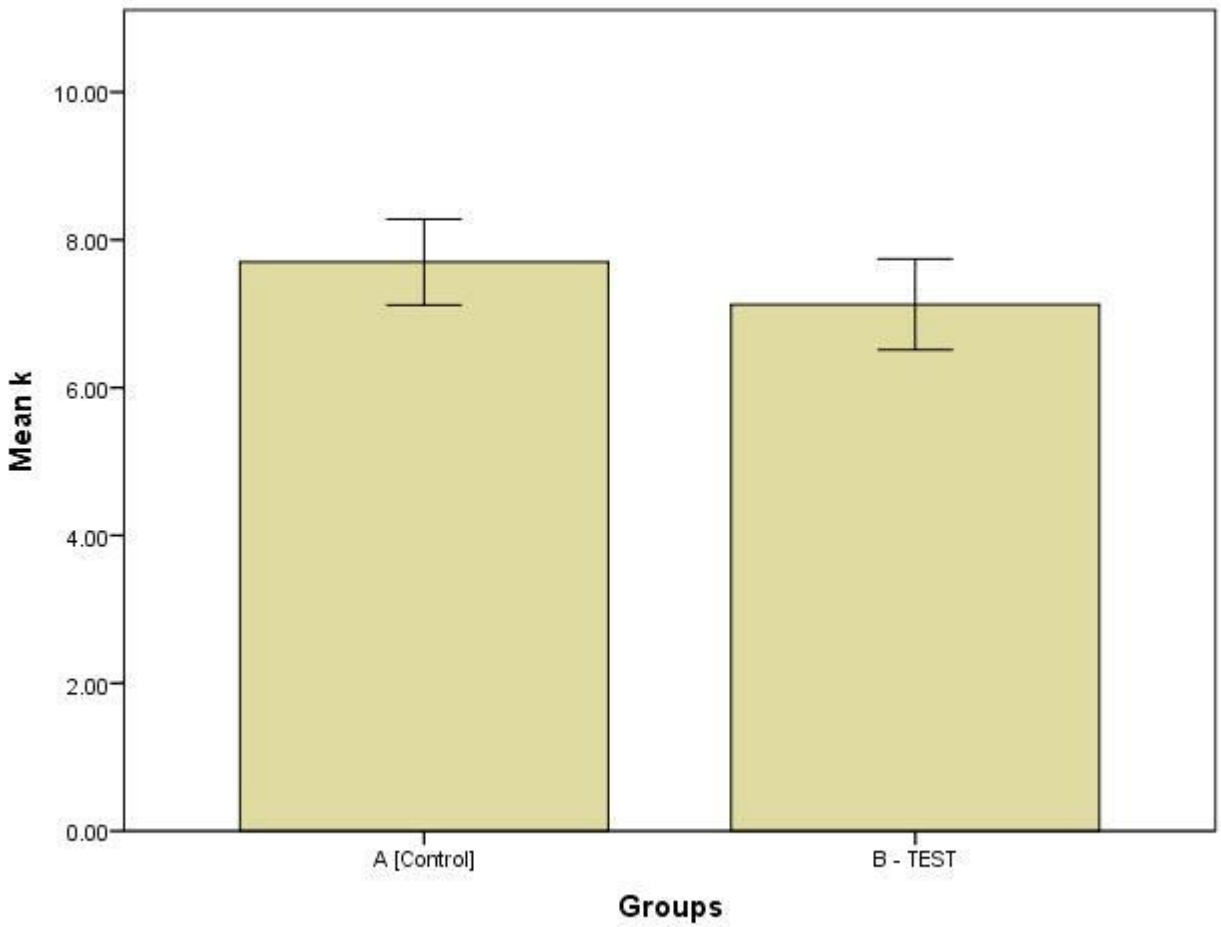
Bicarbonate mean value of wistar rat. Appendix 1e



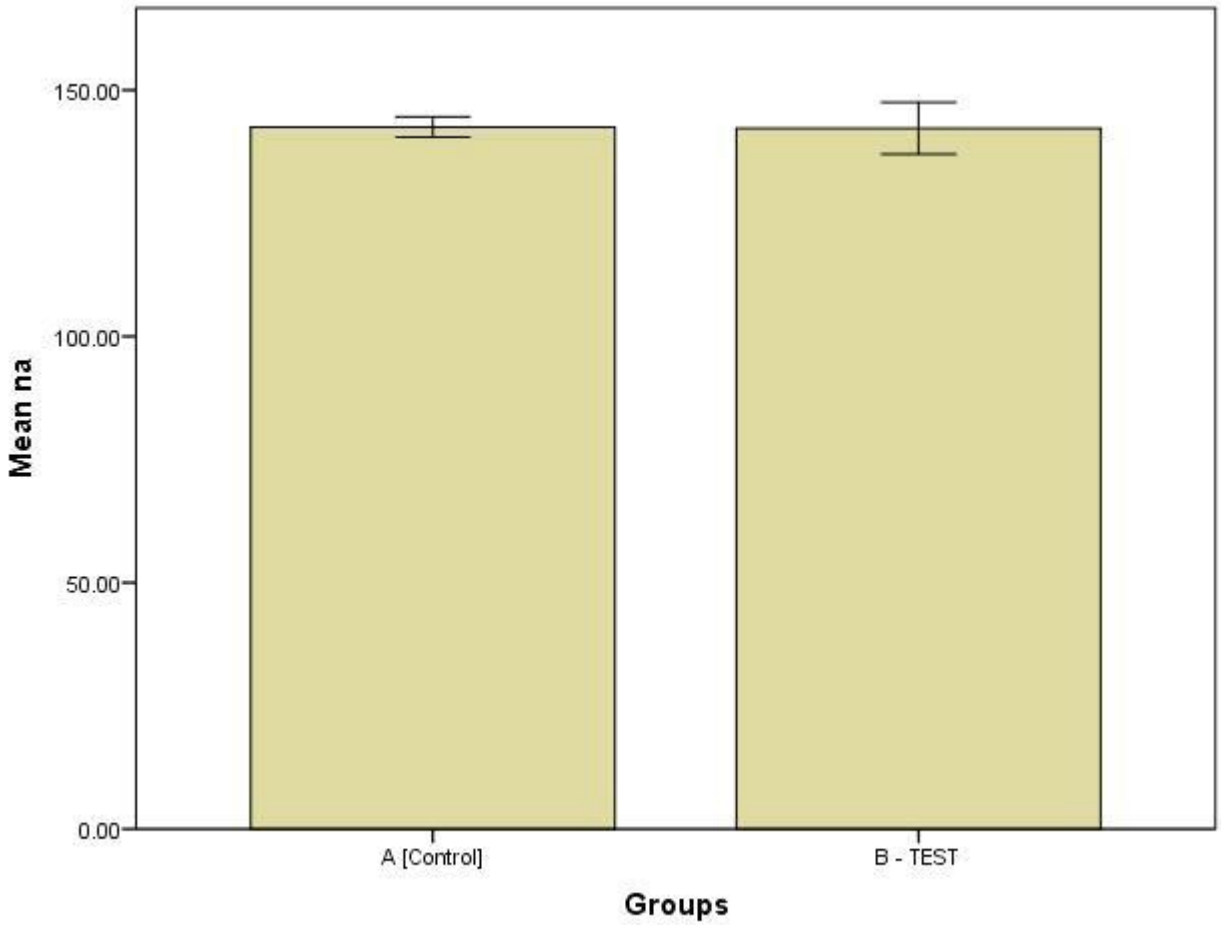
Calcium mean value of wistar rat. Appendix 1f

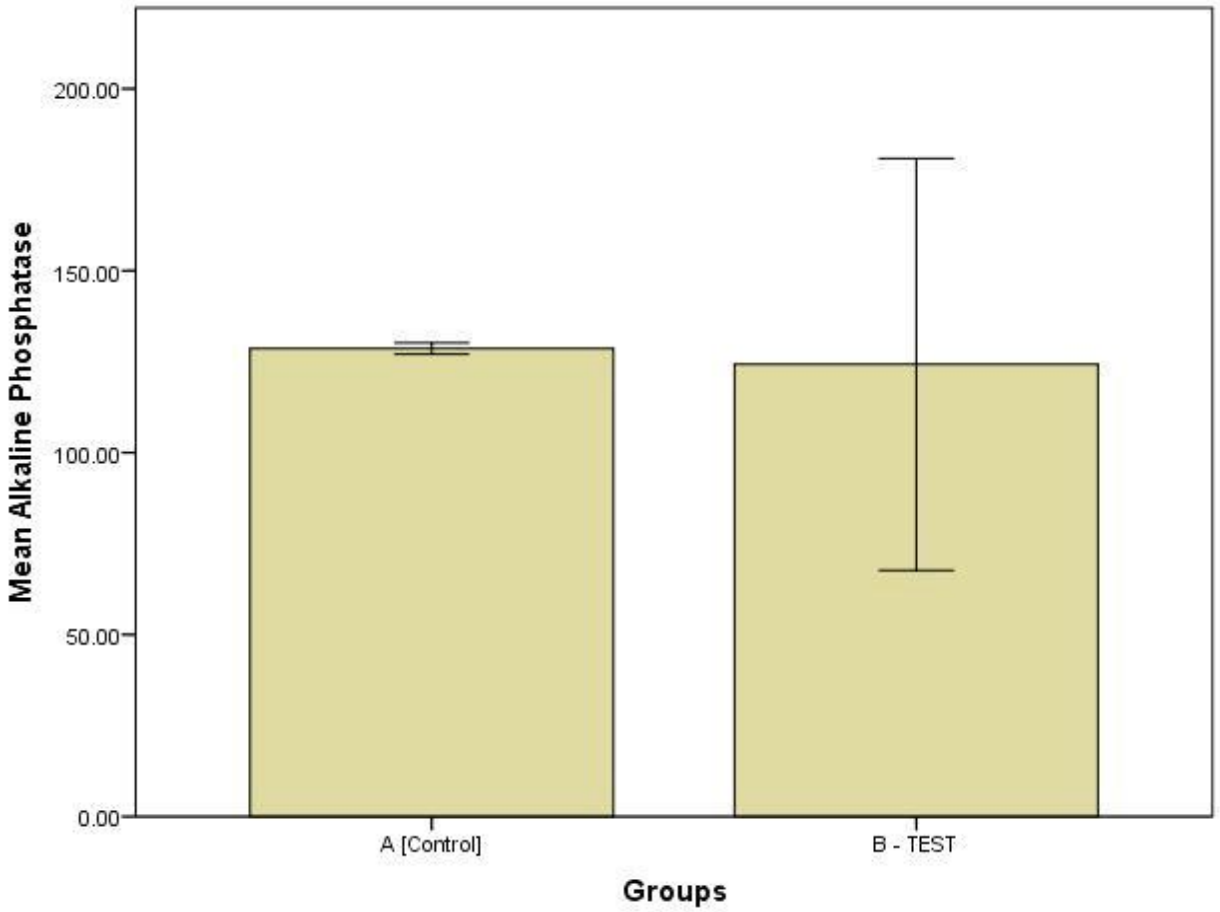


Chloride mean value of wistar rat. Appendix 1g



Potassium mean value of wistar rat. Appendix 1h


150.00
100.00
50.00
0.00
Mean na
A [Control]
B - TEST
Groups


Mean Alkaline Phosphatase
200.00
150.00
100.00
50.00
0.00
A [Control]
B - TEST
Groups

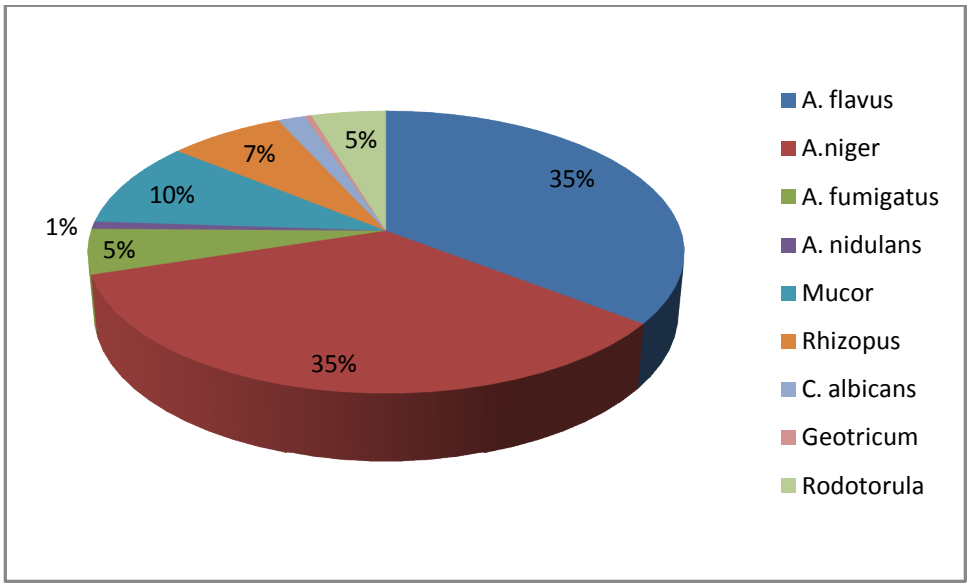


Fig.4.1: General Distribution of Fungi Isolates in Surface Disinfected Cashew Nuts

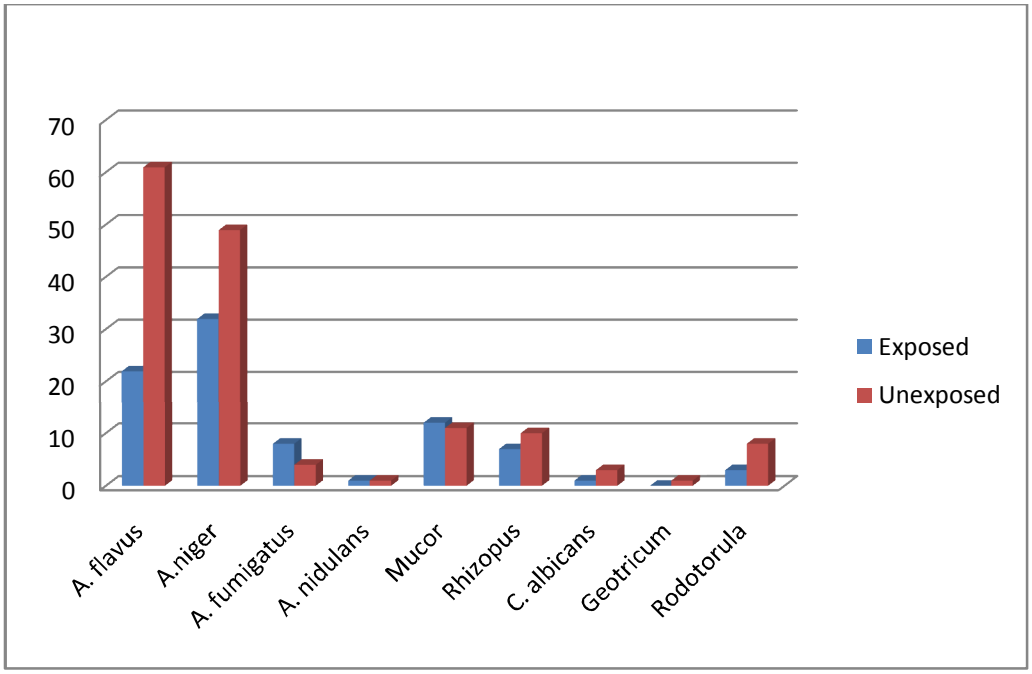


Fig.4.2: ***General Distribution of Fungi Isolates In Exposed And Unexposed CashewNuts***

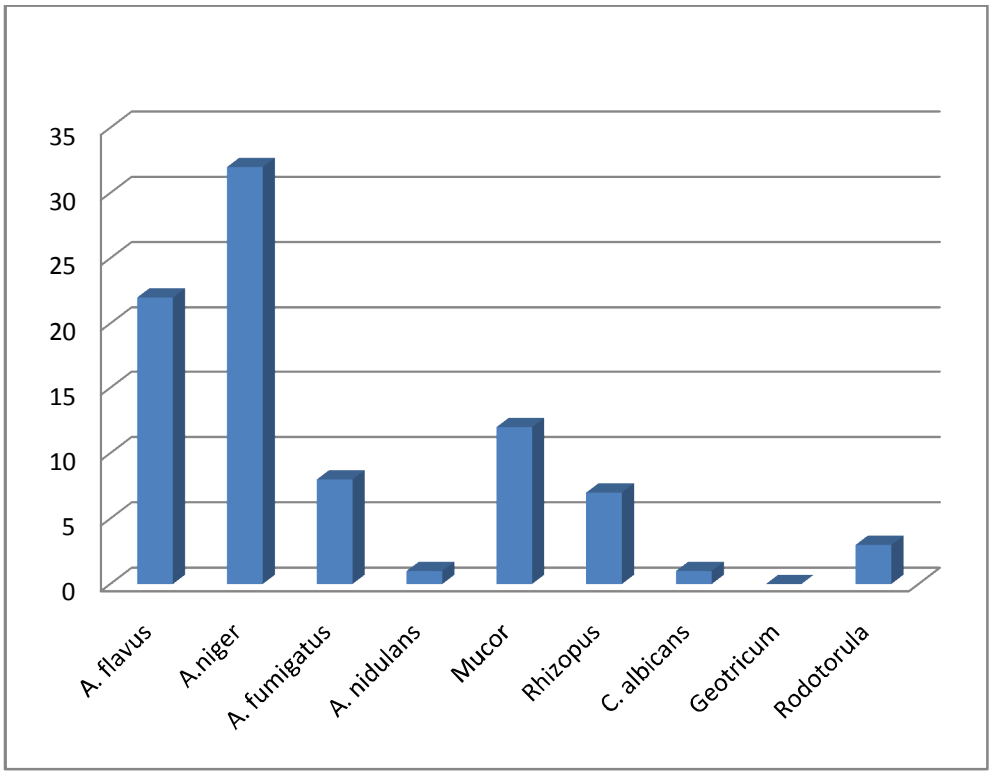


Fig. 4.3: Distribution of fungi contamination of exposed cashew nut

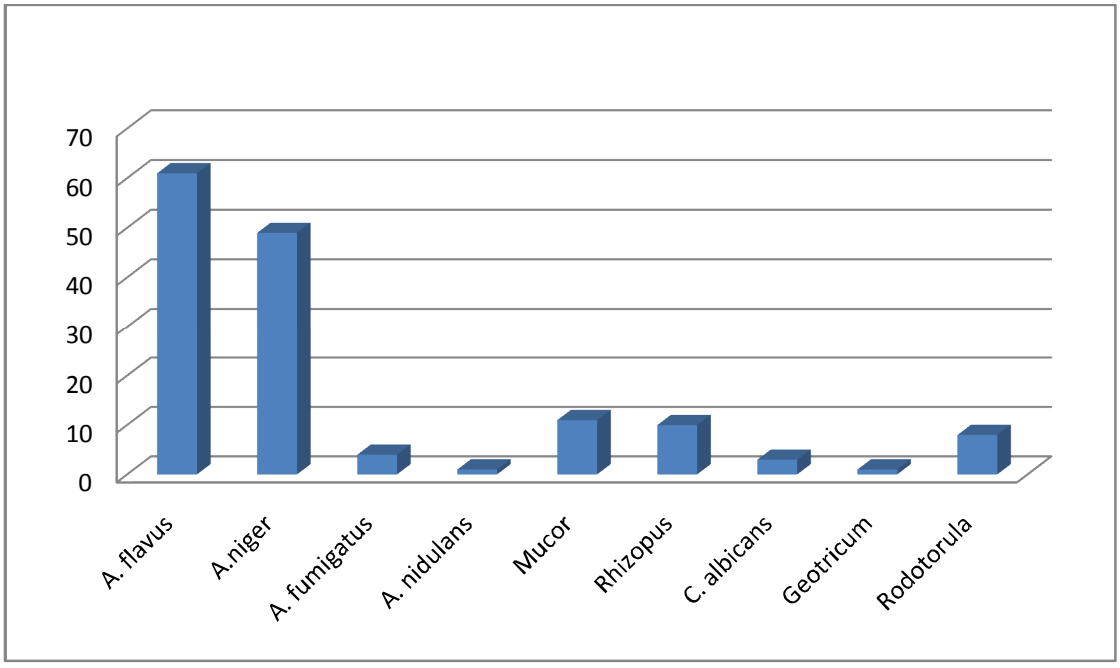


Fig.4.4: Distribution of fungi contamination of unexposed cashew nut

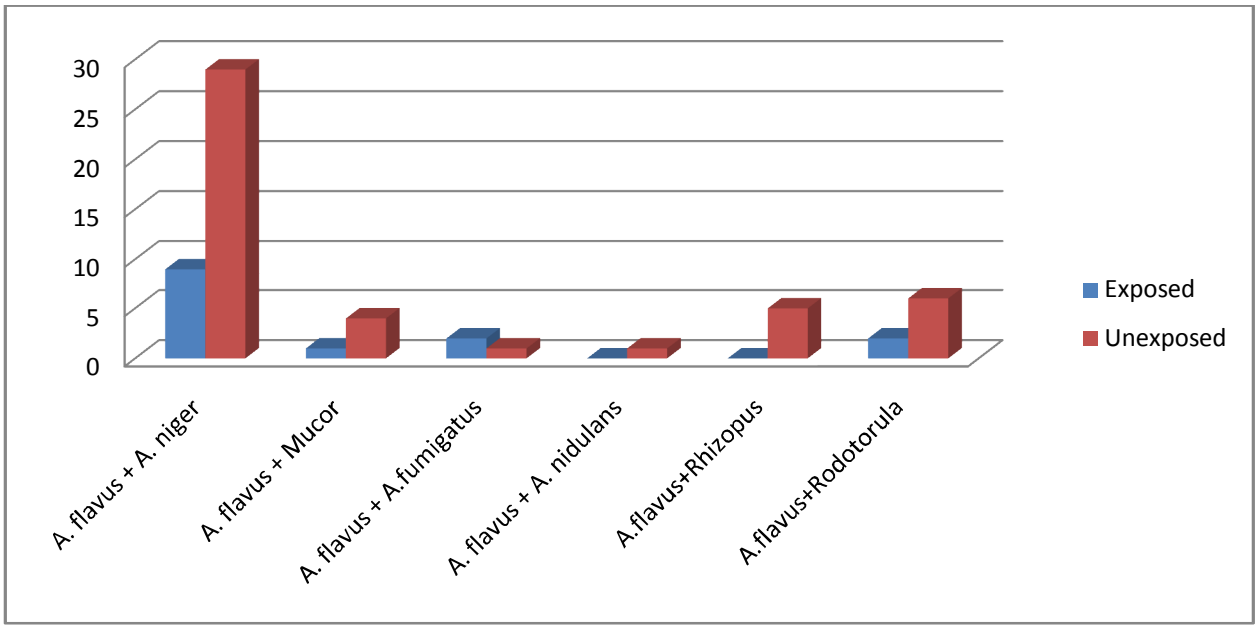


Fig.4.5: Distribution of Aspergillus Flavus Mixed Growth

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 3 data tables and 0 info tables:

---

t test of Variation of Group Fungal Contamination

	Data Set-A
Table Analyzed	Data 1
Column A	Exposed
vs	vs
Column B	Unexposed
Unpaired t test	
P value	0.4185
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8305 df=16
How big is the difference?	
Mean SEM of column A	9.556 3.640 N=9
Mean SEM of column B	16.44 7.454 N=9
Difference between means	-6.889 8.295
95% confidence interval	-24.47 to 10.70
R square	0.04132
F test to compare variances	
F,DFn, Dfd	4.192, 8, 8
P value	0.0585
P value summary	ns
Are variances significantly different?	No

1way ANOVA of A. flavus:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	A. flavus				

One-way analysis of variance					
P value	0.0125				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	9				
F	5.107				
R square	0.8195				
	SS	df	MS		
ANOVA Table	4235	8	529.4		
Treatment (between columns)	933.0	9	103.7		
Residual (within columns)	5168	17			
Total					
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A. flavus vs A.niger	1.000	0.1389	No	ns	-39.28 to 41.28
A. flavus vs A. fumigatus	35.50	4.931	No	ns	-4.781 to 75.78
A. flavus vs A. nidulans	40.50	5.625	Yes	*	0.2186 to 80.78
A. flavus vs Mucor	30.00	4.167	No	ns	-10.28 to 70.28
A. flavus vs Rhizopus	33.00	4.584	No	ns	-7.281 to 73.28
A. flavus vs C. albicans	39.50	5.486	No	ns	-0.7814 to 79.78
A. flavus vs Geotricum	41.00	5.695	Yes	*	0.7186 to 81.28
A. flavus vs Rodotorula	36.00	5.000	No	ns	-4.281 to 76.28
A.niger vs A. fumigatus	34.50	4.792	No	ns	-5.781 to 74.78
A.niger vs A. nidulans	39.50	5.486	No	ns	-0.7814 to 79.78
A.niger vs Mucor	29.00	4.028	No	ns	-11.28 to 69.28
A.niger vs Rhizopus	32.00	4.445	No	ns	-8.281 to 72.28
A.niger vs C. albicans	38.50	5.348	No	ns	-1.781 to 78.78
A.niger vs Geotricum	40.00	5.556	No	ns	-0.2814 to 80.28
A.niger vs Rodotorula	35.00	4.861	No	ns	-5.281 to 75.28
A. fumigatus vs A. nidulans	5.000	0.6945	No	ns	-35.28 to 45.28
A. fumigatus vs Mucor	-5.500	0.7639	No	ns	-45.78 to 34.78
A. fumigatus vs Rhizopus	-2.500	0.3472	No	ns	-42.78 to 37.78
A. fumigatus vs C. albicans	4.000	0.5556	No	ns	-36.28 to 44.28
A. fumigatus vs Geotricum	5.500	0.7639	No	ns	-34.78 to 45.78
A. fumigatus vs Rodotorula	0.5000	0.06945	No	ns	-39.78 to 40.78
A. nidulans vs Mucor	-10.50	1.458	No	ns	-50.78 to 29.78
A. nidulans vs Rhizopus	-7.500	1.042	No	ns	-47.78 to 32.78
A. nidulans vs C. albicans	-1.000	0.1389	No	ns	-41.28 to 39.28
A. nidulans vs Geotricum	0.5000	0.06945	No	ns	-39.78 to 40.78
A. nidulans vs Rodotorula	-4.500	0.6250	No	ns	-44.78 to 35.78
Mucor vs Rhizopus	3.000	0.4167	No	ns	-37.28 to 43.28
Mucor vs C. albicans	9.500	1.320	No	ns	-30.78 to 49.78
Mucor vs Geotricum	11.00	1.528	No	ns	-29.28 to 51.28
Mucor vs Rodotorula	6.000	0.8334	No	ns	-34.28 to 46.28

Rhizopus vs C. albicans	6.500	0.9028	No	ns	-33.78 to 46.78
Rhizopus vs Geotricum	8.000	1.111	No	ns	-32.28 to 48.28
Rhizopus vs Rodotorula	3.000	0.4167	No	ns	-37.28 to 43.28
C. albicans vs Geotricum	1.500	0.2083	No	ns	-38.78 to 41.78
C. albicans vs Rodotorula	-3.500	0.4861	No	ns	-43.78 to 36.78
Geotricum vs Rodotorula	-5.000	0.6945	No	ns	-45.28 to 35.28

Contingency of A. flavus Contamination

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	A. flavus Contamination		
Fisher's exact test			
P value	0.0025		
P value summary	**		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	Yes		
Strength of association			
Relative Risk	1.651		
95% confidence interval	1.163 to 2.343		
Odds ratio	2.708		
95% confidence interval	1.426 to 5.144		
Difference between proportions			
Fraction of top, bottom row in left column	0.6190, 0.3750		
Difference between fractions	0.2440		
95% confidence interval of difference	0.08882 to 0.3993		
Data analyzed	A.flavus contaminated	Non-flavus	Total
Unexposed	65	40	105
Exposed	24	40	64
Total	89	80	169