THE EFFECT OF ORAL INGESTION OF *FUSARIUM OXYSPORUM* INOCULATED SORGHUM ON THE HISTOLOGY OF KIDNEY AND LIVER OF ADULT WISTAR RATS (RATTUS NOVEGICUS)

By

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DECLARATION

I, Anosike, Victor Ikenna hereby declare that the work in this thesis was carried out by me at the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria, under the supervision of Prof .S.A Ojo and Dr S.S Adebisi. No part of this work has been presented in any previous application for the award of a higher degree or honours. All sources of information are clearly indicated and acknowledged by means of references

Anosike, Victor Ikenna --------------------------- --------------
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Signature Date

CERTIFICATION

The thesis entitled The effect of oral ingestion of *Fusarium oxysporum* contaminated sorghum on the histology of kidney and liver of wistar rats" by Anosike Victor Ikenna meets the regulation governing for the award of the degree of Master of Science (M Sc) in Human Anatomy Of the Ahmadu Bello University and is hereby approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis I dedicate to the living, and loving memory of my lovely big sister,

Ngozichukwu Peace Anosike, courageous and calm even in death.

Slept, sat., 4th Aug.1984

And to my dear parents, Mr. and Mrs. S.N Anosike, whose worth to me can never be estimated.

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My foremost and most profound gratitude goes to the eternal one, my refuge, my fortress, my very present help in trouble and my deliverer.

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To my Teachers, I say thank you. To S.B Danborno (Mallam), thank you for being more than a teacher, thank you for inspiring me.

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ABSTRACT

Fusarium oxysporum is an important pathogen of a wide range of crop plants. The study was conducted to investigate the effect of feeding a blend of sorghum grains contaminated with *Fusarium oxysporum* on the blood picture and the histology of the kidney and liver of adult wistar rats. A total of thirty (30) rats of the wistar

species weighing between 150 and 180g were divided into four experimental groups and a control group comprising six(6) rats in each group and fed for twenty eight days(28).The diet consisted control(group 1),25% contaminated meal(group 2),50% contaminated(group 3),75% contaminated(group 4) and 100% contaminated meals(group 5).

At the end of the experiment the RBC, WBC and differential WBC counts did not show any significant differences ($P>0.05$).the histological studies however showed damage to the kidney tissue under light microscopy. They were characterized by degraded renal tubules, necrosis of glomerular cells, and apoptotic bodies in areas between the renal tubules infiltrated by mononuclear cells.

The liver histology showed by light microscopy, loss of hepatic architecture, pyknotic nuclei and infiltration by mononuclear cells.

It was therefore concluded that *Fusarium oxysporum* has caused marked inflammation and tubular necrosis in the rat's kidney as well as inflammation and parenchymal necrosis in the liver with no significant changes in the blood picture.

TABLE OF CONTENT

Title page……………………………………………………………………………i

Declaration……………………………………………………………………ii

Certification...iii

Dedication..v

Acknowledgement...vi

Abstract..viii

Table of content..ix

List of tables..xiv

List of figures...xv

List of

plates...xvii

List of abbreviations...xix

CHAPTER ONE

INTRODUCTION

1.1 Introduction..1

1.2 Objectives..3

1.3 justifications...4

1.4 statement of problem,,.5

CHAPTER TWO

LITERATURE REVIEW

2.1 Liver positions..6

2.1.1 Liver Development...6

2.1.2 Histology of the Liver...7

2.2 The kidney..8

2.2.1 Internal Structure of the Kidney...9

2.2.2 The Uriniferous Tubule..10

2.2.3 The Nephron..11

2.3 *Fusarium oxysporum*..*11*

2.3.1 Description and natural habitat...13

2.4 Mycotoxins..14

2.4.1 Chemistry and Toxicology of Mycotoxin..............................15

2.4.2 Fusaric acid...16

2.4.3 Moniliformin..16

2.4.4 Fumonisins B1...20

2.4.5 The Trichothecenes..23

2.4.6 Zearalenone..30

2.4.7 Stability of Mycotoxins.. 32

2.4.8 Factors Affecting Production of Mycotoxins........................... 32

2.4.9 Mechanism of Action...34

2.5. Morphology and Functional effects of Fusarium Oxysporum ...36

2.5.1 Cytological Alteration Nucleus B.Mitotic Aberration......... 37

2.5.2 Histological Damage...39

a. liver

b..Kidney

CHAPTER THREE

MATERIALS AND METHODS

3.2.0 Culture Preparation.. 41

3.2.1 Materials...41

3.2.2 Preparation of contaminated Grains.......................................41

3.3.0 Feed Preparation...42

3.4. Feeding Regimen...43

3.5 Hematology..43

3.6. Calculations..44

3.7 Histological Experiment...45

3.7.1 laboratory materials..45

3.7.2 Chemicals...46

3.8. Methodology...46

CHAPTER FOUR

RESULTS

4.2. Hematological Indices...49

4.3 Kidney Pathology..63

4.4 Liver Pathology...65

CHAPTER FIVE

DISCUSSIONS

5.2 Hematology..68

5.3 Direct and Indirect Effects of Fusarium Mycotoxins............69

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1

Conclusion...7

2

6.2 Suggestion for Further Work..72

References...73

LIST OF TABLES

TABLE 1...... Table of effect of dietary *Fusarium Oxyporum* on some hematological parameters..50

TABLE 2 The effect of dietary Fusarium Oxysporum on total white blood cells..51

TABLE 3 The effect of dietary Fusarium Oxysporum on Neutrophil, Lymphocyte,Monocyte,Basophil and Eosinophil............................52

LIST OF FIGURES

Fig 1 Structural formula of fusaric acid..17

Fig 2 Structural formula of monoliformine.................................18

Fig 3 Structural formula of fumonisinB_1..21

Fig 4 Structural formula of type A trichothecene..........................23

Fig 5 Structural formula of type B trichothecene..................24

Fig 6 Structural formula of T-2 TOXIN...................................25

Fig 7 Structural formula of Diacetoxyscirpenol.................................26

Fig 8 Strucrual formula of deoxynivalenol (VOMITOXIN)....................27

Fig 9 Structural formula of zearalenone...30

LIST OF PLATES

Plate 1 T.S. of kidney of rat in group 1(control)......................................53

Plate 2 T.S of kidney of rat in group 2 (experimental)............................54

Plate 3 T.S. of kidney of rat in group 3 (experimental)..............................55

Plate 4 T.S. of kidney of rat in group 4 (experimental)............................56

Plate 5 T.S. of kidney of rat in group 5 (experimental)............................57

Plate 6 T.S. of the Liver of rat in group 1(control).................................58

Plate 7 T.S. of the Liver of rats in group 2 (experimental).......................59

Plate 8 T.S. of the Liver of rats in group 3 (experimental).........................60

Plate 9 T.S. of the Liver of group 4 (experimental)................................61

Plate 10 T.S. of the Liver of group 5 (experimental)...............................62

List of abbreviations

RBC=Red blood cell

Neutr. =Neutrophil

Basoph. =Basophil

Conta. =Contaminated

WBC=White blood cell

Mono=Monocytes

Eosino=Eosinophil

Lymph=Lymphocytes

T-2: = T-2 Toxin

HT-2 =HT-2 Toxin

DON = Deoxynivalenol

NIV = Nivalenol

3-AcDON =3-Acetoxynivalenol

15-AcDON = 15-Acetoxynivalenol

FUS-X = Fusarenon-X

DAS=Diacetyloxyscirpenol

Grp= Group

CHAPTER ONE

1.1 INTRODUCTION

The word mycotoxin derives from the Greek 'mykos' (fungus) and the Latin 'toxicum' (Poison) (Kenji and Mikio,1978), and is at present used to designate secondary fungal metabolites which cause pathological changes, or physiological abnormalities in man and warm blooded animals (Kenji and Mikio,1978). These Compounds are produced by the fungi in their mycelium, but may also be present in the spore of the organism. The deleterious effects are referred to as mycotoxicosis (D' Mello and MacDonald 1997). Mycotoxins are generally produced after a period of balanced growth. The production of particular mycotoxin is however confined to a relatively small number of fungal species, and may even be strain specific. There is convincing evidence to suggest that the more intricate the pathway of synthesis of a particular mycotoxin, the fewer the number of fungi producing that compound (D' mello and Macdonald 1997;Smith 1997).

The Untold effect of moulds and fungi was known already in ancient times. In the seventh and eighth centuries BC, the festival 'Robigalia' was established to honour the god Robigus, who had to be propitiated in order to protect grain and tree. It was celebrated on the

25th of April, because that was the most likely time for crops to be attacked by rust or mildew (Peraica et al., 1999).

In the middle ages, outbreaks of egotism caused by ergot alkaloids from clariceps purpurea reached epidemic proportions, mutilating and killing thousands of people during the feudal days in Europe. This disease was known as "ST Anthony's fire" or "Holy fire", because at that time it was believed that a pilgrimage to the shrine of ST Anthony would bring relief from the intense burning sensation experienced (Kenji and Mikio,1978;Peraica et al. ,1999).

Two characteristic forms of the poisoning were described; gangrenous and Convulsion egotism. The victims of egotism were exposed to lysergic acid diethylamide (LSD) a hallucinogen, produced during the baking of bread made with ergot contaminated wheat (Peraica et al.,1999).

Fusarium – mediated toxicoses represents the most typical, but complex mycotoxicosis so far known. From a historical viewpoint, an important report was published in 1891 by Woronin, describing in details, the 'Tau – melgetreide' (Staggering grain) intoxication that affects man and domestic animals , and *Fusarium roseum* as causal fungi (Kenji and Mikio,1978). Similar poisoning due to mouldy cereals and their products also occurred widely in the former USSR, central Europe,

Finland, North America, China and other districts where wheat, barley, millet or corn was consumed as staple food. When the infected or mouldy cereals are consumed, serious disease may develop in man and farm animals such as Vomiting, diarrhoea, feed refusal, Congestion or haemorrhage in the lungs, adrenal, intestine, uterus, vagina and brain, and destruction of bone marrow are the characteristic symptoms (Tsunoda 1970;).

1.2. OBJECTIVES

In the absence of hard data on mycotoxins present in fusarium oxysporum, as well as histological studies showing its association with damage in GIT organs and viscera, the present study was conceived with the primary objectives of:

Determining the effect of oral consumption of grain contaminated with *Fusarium oxysporum* on the histological parameters of the Liver and Kidney of adult wistar rats .

- Determining such effects on a sub chronic level.
- Making deductions as for the possible aetiology of these factors with a view to explaining the possible toxic agent contained.
- Drawing attention to the dangers of consuming fungi contaminated foods.

- Spring more research interest into environment microbes as etiological factors in the development and enhancement of disease, especially in our rural communities.

1.3 JUSTIFICATION

1. Since the last century, following upon outbreaks that occurred in Europe and Asia after the II world war, much interest and research focused on mycotoxins to the extent that their biological effect on man and animals have been elicited. Now regulatory issues are well defined for many of these places with regard to mycotoxins except tropical Africa. This work it's hoped will stimulate government to make regulations guiding the limits of mycotoxins permissible in cereals for local consumption.

2. In order to conclude that a certain mass disease of unknown aetiology is actually a human mycotoxicosis, it is necessary to establish definitely that the agent acting to cause disease in man is in fact mycotoxic in nature. Inducing such mycotocosis in man for comparative purposes is of course impermissible. The only course open would be to reconstruct the circumstances surrounding outbreaks while etiological studies are carried out especially from the viewpoint of toxicological studies in animals. This study it is hoped will begin to answer the question of

possible factors mediating strange ailments especially in rural communities.

3. Cereals are the most important source of human food. It is estimated that 10 – 30% of the harvested grains is lost due to mould infection (Chelkowski, 1991). Usually and especially in developing countries, the best quality cereals are exported while the crop with poorer quality is consumed in the homeland (Dawson ,1991). Hence the citizens in developing countries, mainly living in rural areas are especially sensitive to adverse health effects of mycotoxins due to malnutrition and low standard of living (Shane ,1994). Perhaps this study will show the extent of damage this condition could elicit

1.4 STATEMENT OF PROBLEM

Sorghum forms one of the staple food of people living in Zaria and environ. Often at harvest time, these grains are left for too long in the field, allowing enough time for the fungi to develop their attendant mycotoxicosis. Perhaps this could explain the several cases of kidney and liver diseases that characterizes the rural communities

CHAPTER TWO

LITERATURE REVIEW

2.1 LIVER :POSITION

The liver, the largest gland in the human body weighs approximately 1500gms, and accounts for approximately one -fortieth of the adult body weight. It lies in the right and left upper quadrants (mainly on the right side) inferior to the diaphragm which separates it from the pleura, lungs, pericardium and heart (Moore and Dalley, 1999).

2.1.1 LIVER :DEVELOPMENT

The liver develops from endodermal bud that arises from the ventral aspect of the gut, at the point of junction between the foregut and midgut. The bud grows into the ventral mesogastrium and passes through it into the septum transversum.. It enlarges and soon shows divisions into a larger cranial part called 'pars hepatica', and a smaller caudal part called ' pars septica".Pars hepatica divides into right and left parts , each of which form one lobe of the liver(Williams et al., 2004).

As the right and left divisions of the pars hepatica enlarge and extend into the septum transversum, the cells arising from them are broken up into interlacing columns called trabeculae. In this process, the

umbilical and vitelline veins which lie in the septum transversum are broken up to form the sinusoids of the liver. Sinusoids are also formed from the mesenchyme of the septum transversum(Williams et al., 2004).

The endodermal cells of the hepatic bud give rise to the parenchyma of the liver, and to bile capillaries. The mesoderm of the septum transversum forms the capsule and fibrous tissue basis of the liver(Williams et al., 2004).

2.1.2 HISTOLOGY OF THE LIVER

The liver may be regarded as a modified exocrine gland that has other functions. It is made up, predominately of liver cells or hepatocytes. Each hepatocyte is a large cell with a round open faced nucleus with prominent nucleoli(Ham, 1974).

The liver substance is divisible into a large number of large lobes, each of which consists of numerous lobules. The exocrine secretion of the liver is called bile. Bile is poured out from liver cells into very delicate bile canaliculi that are present in an intimate relation with the cells. From the canaliculi, bile drains into progressively larger ducts which end in the bile duct(Ham, 1974).

In sections through the liver, the substance of the organ appears to be made up of hexagonal areas that constitute the hepatic lobules. In the

human liver, the connective tissues septa demarcating the lobules are scanty unlike in pigs. In transverse section, each lobule appears to be made up of cords of liver cells that are separated by sinusoids. However the cells are really arranged in the form of plates, one cell thick that branch and anastomose with one another to form a network. Spaces within the network are occupies by sinusoids .

Along the periphery of each lobule there are angular intervals filled by connective tissues. These intervals are called portal canals. The canals forming a connective tissue network permeate the entire liver substance. Each canal contains:

A branch of the portal vein

A branch of the hepatic artery

An interlobar bile duct.

These three structures form a portal triad.

The cytoplasm of liver cells contain numerous mitochondria, abundant rough and smooth endoplasmic reticulum, a well developed Golgi complex,lysosomes and vacuoles containing various enzymes. Numerous free ribosomes are present also. These features are to be correlated with the high metabolic activity of liver cells. Stored glycogen, lipids and iron (as crystals of ferratin and haemosiderin) are usually present(Ham,1974).

2.2. THE KIDNEY

The ovoid kidney lies peritoneally on the posterior abdominal wall, one on each side of the vertebral column at the level of T12 through L 3 vertebrae. The right usually lies slightly inferior to the left kidney because of the large size of the right lobe of the liver. During life they are both reddish brown and approximately 10cm in length,5cm in width and 2.5cm in thickness(Moore and Dalley, 1999).

At the concave medial margin of each kidney there is a vertical cleft, the renal hilum,where the renal artery enters and the renal vein and renal pelvis leave the renal sinus

The ureters are muscular ducts 25-30cm long, with narrow lumina that carry urine from the kidney to the urinary bladder. The apex of the renal pelvis is continuous with the ureter(Moore and Dalley, 1999).

2.2.1 INTERNAL STRUCTURE OF KIDNEY

The internal structure of the kidney can be seen when we examine a coronal section of the organ. Kidney tissue consists of an outer part called the cortex and an inner part called the medulla(Williams et al.,2004).

The medulla is made up of triangular areas of renal tissue that are called the renal pyramids. The base of each pyramid is directed towards

the cortex and its apex towards the renal pelvis, and fits into a minor calyx. The pyramid shows striations that pass radially towards the apex(Williams et al.,2004).

The renal cortex consists of the following:

- Tissue lying between the bases of the pyramid and the surfaces of the kidney forming the cortical arches or the cortical lobules. This part of the cortex shows dark light lines, the lines are the medullary rays.
- Tissue lying between adjacent pyramids is also a part of the cortex, this constitutes the renal column.

In this way each pyramid is surrounded by a shell of cortex. The pyramid and the cortex around it constitute a lobe of the kidney(Moore and Dalley,1999).

2.2.2 THE URINIFEROUS TUBULE

Each uriniferous tubule consists of an excretory part called the nephron, and a collecting tubule. The collecting tubule draining different nephrons joins to form larger tubules called the papillary ducts (of Bellini), each of which opens into a minor calyx at the apex of a renal papilla. Each kidney contains one or two million nephrons(Williams et al., 2004).

2.2.3 THE NEPHRON

The nephron consists of a renal corpuscle (Malpighian corpuscle) and a long complicated renal tubule. The renal corpuscles consist of blood capillaries called glomerulus and a double layered covering called the glomerulus capsule (Bowman's capsule). The urinary space between the capsules continues with the lumen of the renal tubule(Ham, 1974).

The renal tubule is divisible into:

(A) Proximal convoluted tubule

(b) The loop of Henley

(c) The distal convoluted tubule.

Renal corpuscle and the greater parts of the proximal and distal convoluted tubules are located in the cortex. The loops of Henley and collecting ducts lie in the medullary rays and in the substance of the pyramids(Ham, 1974).

2.3 FUSARIUM OXYSPORUM

Fusarium is a large genus of filamentous fungi, widely distributed in soil and in association with plants. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. Some species produce mycotoxins in cereal crops that can affect human and animal health if it enters the food chain. The main toxins produced by

these fungi are fumonisins and tricothecenes (Marassas and Nelson,1984)

The Mycotoxins of *Fusarium oxysporum* are T2 and HT -2 Toxins, also diacetoxyscirpenol (DAS) (Mirocha and Abbas,1989). T -2 toxins are known to produce cytotoxic effects by inducing lipid peroxidation via the generation of free radicals (Rizzo et al .,1994). These toxins result normally in grains after water damage to the grains such as occurs when it remains for extended periods in the fields or after harvest, especially in cold weather, or in grain that become wet during storage. (Canady et al., 2001).

The trichothecenes are known to affect organs, especially of the liver, digestive tract, kidney, lungs, thymus and heart (Kenji and Mikio, 1978). Deoxynivalenol (DON) a toxic mycotoxin (Commonly called Vomitoxin) produced by *Fusarium graminearum* did not cause significant lesions or abnormalities in the liver, spleen , hearts and kidneys of pigs fed on wheat containing it (Pollman et al.,1985). *Fusarium graminearium* has however been reported to produce T -2 toxin which is over 400 – fold more toxin than DON, and DAS (Atroshi et al., 1997). This pathogen has been shown to cause inflammation and parenchyma damage in the rat liver, as well as inflammations and tubular damage in the kidney (Ozbek and Ozbek,2003).

2.3.1 DESCRIPTION AND NATURAL HABITAT

Fusarium oxysporum has the below taxonomical classification

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Hypocreaceae
Genus	*Fusarium*
Specie	*oxysporum*(Walsh, 1996;(Marassas

and Nelson,1984)

The colony of *Fusarium oxysporum* is usually purple or pale coloured; micronidia are ellipsoid sometimes curved, and produced in slimy heads rather than chains. The phialides producing micronidia are often quite short and broad, proliferating forks at the tip. The macronidia are rather sharply pointed and hooked over at the apex. Chlamydospores often found, are seldom abundant (Nelson et al., 1983).

Hyaline septate hyphae, phialides, micronidia and Chlamydospores can be observed microscopically. Phialides are cylindrical with a small

collavette, solitary or produced as part of a complex branching system. Micronidia are produced from phialides.

2.4 MYCOTOXINS

Fungi which are capable of producing toxic metabolites are distributed over a large number of fungal families and no particular rule or principle exists concerning the ability to produce mycotoxins. Further, because the biological and chemical nature of mycotoxins is highly diverse, it is difficult to distinguish clearly between types of fungal metabolites that are toxic and non toxic. Mycotoxins have been classified into Penicillium mycotoxins, Aspergillus mycotoxins, Fusarium mycotoxins and miscellaneous mycotoxins(Kenji and Mikio,1978).

Mycotoxins reported from *Fusarium oxysporum* include:

Diacetoxyscirpenol

Diacetylnivalenol

Fumonisins B_1

Fusarenon – x

Fusaric acid

Moniliformin

Neosolaniol

T2 Toxin

Zearalenone (Marassas and Nelson,1984).

Mycotoxins are the toxic chemical produced by fungi for a variety of reasons. These include to attack or gain access to host by helping to dissolve cell membranes, or as a protective measure against encroaching organisms. The production of mycotoxins within the fungus depends on food source, and the particular enzymes of the fungus, and other environmental factors. Usually, mycotoxins are not found in the spores, but generally produced in the next stage, that of the mycelium. Fungi can however produce different toxins and varying amount of toxins depending on which media they are growing on, humidity, temperature and light among other variables (Marassas and Nelson,1984).

2.4.1 CHEMISTRY AND TOXICOLOGY OF MYCOTOXINS

Mycotoxins are relatively stable compounds mostly with low molecular weight. Several of them are generally lipophilic and accumulate in the fat fraction of plants and animals (Hussein and Brassel,2001).

2.4.2 FUSARIC ACID

Fusaric acid, 5-butyl picolinic acid, is a mycotoxin with a low or moderate toxicity, which is of concern since it might be synergistic with other co-occurring mycotoxins. It has profound effects on the cardiovascular system as a potent inhibitor of dopamine β hydroxylase in vivo and invitro (Nagatsu et al.,1970). intraperitoneal injection of 20mg/kg in rabbits, rats, cats, and dogs produced profound and persistent decrease in arterial pressure because of decrease in endogenous nor-epinephrine concentration (Nagatsu,1970) . The systemic hypotensive response to fusaric acid is comparable to that observed in pigs fed culture materials containing fumonisins (Smith et al.,1996). The hypotensive effect of fusaric acid was once being investigated as an antihypertensive drug in human (Terasanda and Komeyama ,1971).

2.4.3 MONILIFORMIN

Moniliformin (MON) is the trivial name for 3-hydroxy-3 Cyclobutene- 1, 2, dione, an anion usually occurring with potassium or sodium as a counter –ion (K/Na CAs[5259-22.7]KorNaC4HO3, MW = 136.11g/mol(Joseph,1999;Sewram et al.,1999)

It is a mycotoxin produced by fusarium species (mainly T.Fujikurio, F.Proliferatum, avenacium, subglutinans). MON is toxic acid phytotoxic *Fusarium Moniliformine* isolate exhibit cancer promoting activity on short

term cancer initiation promotion bioassay with diethyl nitrosamine initiated rats (Lemmer et al., 1999).

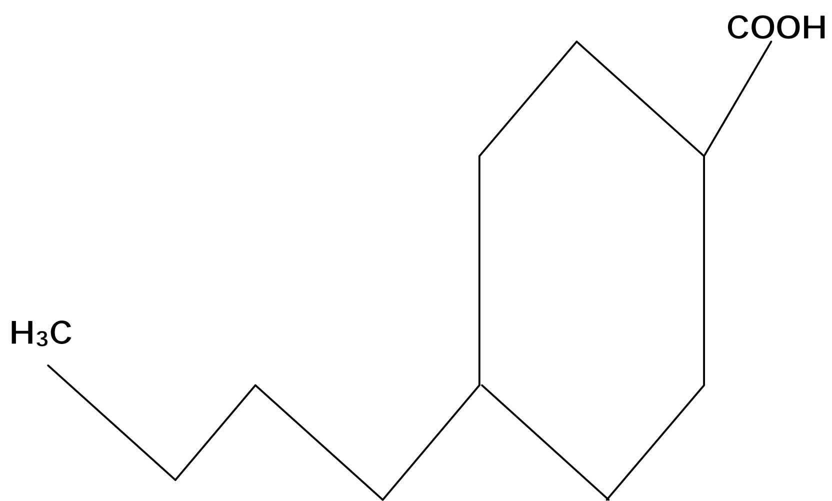


Fig 1 Structural formula of fusaric acid

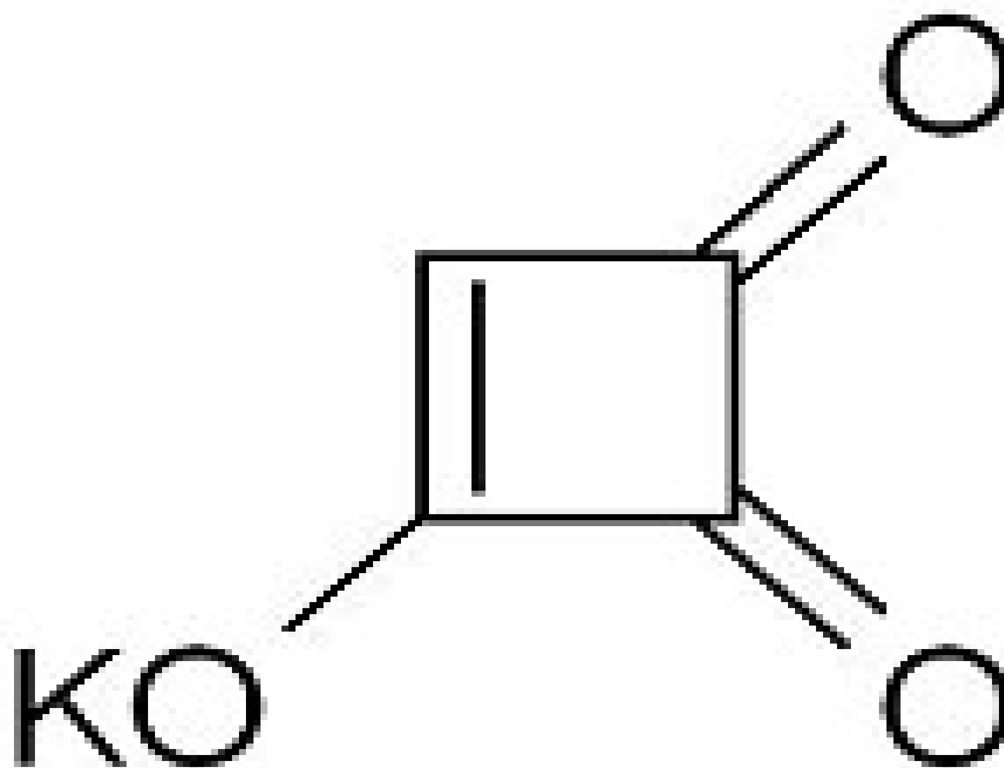


Fig 2 Structural formula of monoliformin

2.4.4 FUMONISINS B_1

Fumonisins B_1 is the diester of propane 1,2,3, tricarboxylic Acid and 25-animo- 125, 16R-dimethyl 35, 5R, 10R, 14s, 15R- Pentahydroxy eicosane in which the C-14 and C-15 hydroxyl groups are esterified with the terminal carboxyl group of propane 1,2,3 tricarboxylic acid .Fumonisins B_1 is the most prevalent of the mycotoxins produced by *Fusarium Moniliforme* (Gelderbom et al.,1988).Outbreaks of acute food borne diseases possibly caused by fumonisins has been reported in India. The individuals attacked were from the poorest social strata and had consumed maize and sorghum harvested and left in the fields during unseasonable rains (Peraica et al., 1999). The incidence and concentration of aflatoxin, deoxynivalenol and fumonisins were recently determined in maize samples from an area of China with a high incidence of primary liver cancer, and from an area with a low incidence. Aflatoxin was found in low concentration in almost all maize samples from both areas, but the incidence and concentration of deoxynivalenol and fumonisins were much higher in the sample from the area were incidence of primary liver cancer was high. The authors put forward the hypothesis that fumonisins which have known cancer promoting activity in rat liver, and deoxynivalenol promote the initial lesion caused by aflatoxin. Toxins from *Fusarium moniliforme* have therefore been classified as possible carcinogenic substance to human (Peraica et al.,

1999). They are also known to cause hepatic lesions in pigs and cattle, equine encephalomalacia, porcine primary oedema and oesophageal cancer in humans (D'Mello, 1997).

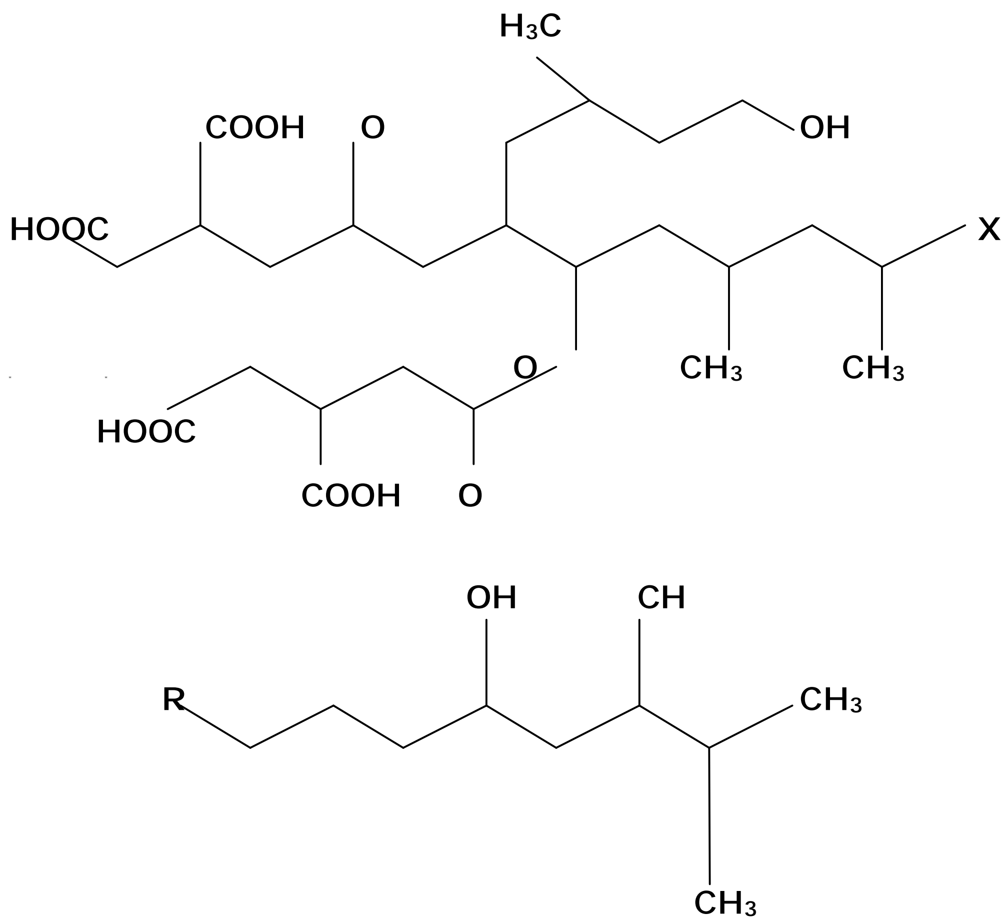


Fig 3 Structural formula of fumonisin B_1

2.4.5 THE TRICHOTHECENES

Trichothecenes are mycotoxins produced mostly by members of the Fusarium genus. About 148 trichothecenes have been isolated, but only a few have been found to contaminate food and feed (Peraica et al., 1999). Trichothecenes are attractive warfare agent because they can enter the body either by way of the skin, by inhalation or by ingestion.

Trichothecenes are tetra-cyclic compounds with a sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system. They are arbitrarily divided into four groups. A, B, C, and D according to their chemical structure (Kenji and Mikio, 1978).

The type A trichothecenes has the carbonyl group in position C-8 missing. The type A trichothecenes includes T-2 toxin, HT-2 toxin, Neosolaniol and diacetoxyscirpenol (DAS). The type B have the carbonyl group in position C-8 and include deoxynivalenol (DON), Vomitoxin, nivalenol and fusarenon-X (D'Mello et al., 1997). The two types of trichothecenes are the major groups. The type C have a second epoxide group and type D trichothecenes are macrocylic compounds.

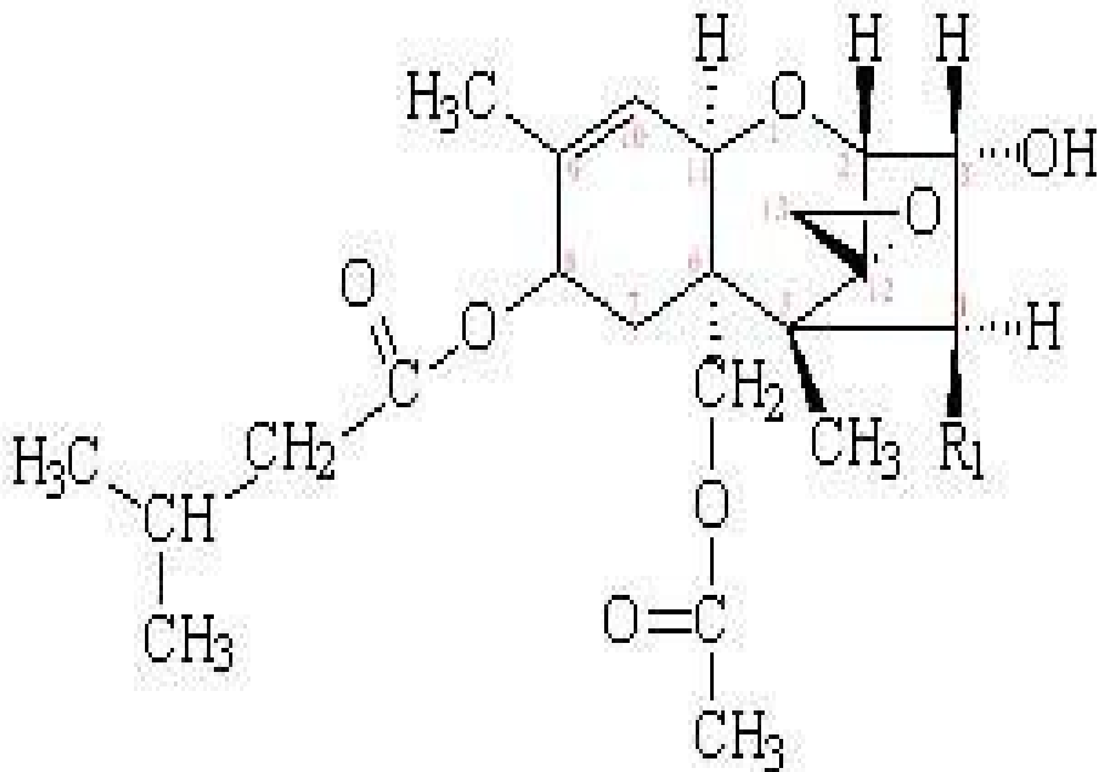


Fig 4 Structural formula of type A trichothecenes

T-2: (R_1 = OAc)

HT-2 (R_1 = OH)

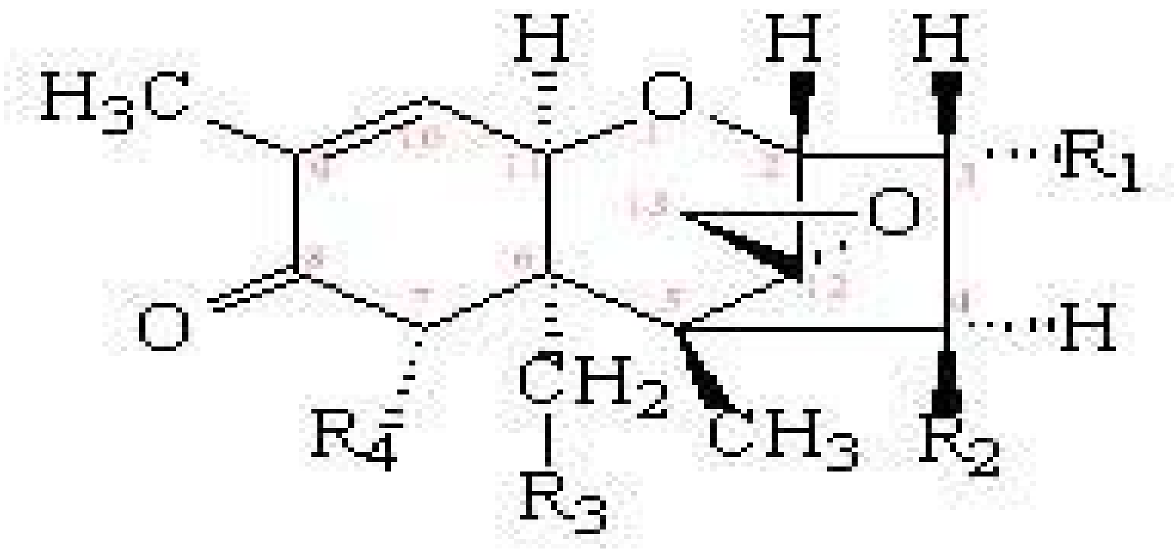


Fig 5 **Structural formula of type B trichothecenes**

DON (R_1 = OH, R_2 = H, R_3 = OH, R_4 = OH)

NIV (R_1 = OH, R_2 = OH, R_3 = OH, R_4 = OH)

3-AcDON (R_1 = OAc, R_2 = H, R_3 = OH, R_4 = OH)

15-AcDON (R_1 = OH, R_2 = H, R_3 = OAc, R_4 = OH)

FUS-X (R_1 = OH, R_2 = OAc, R_3 = OH, R_4 = OH)

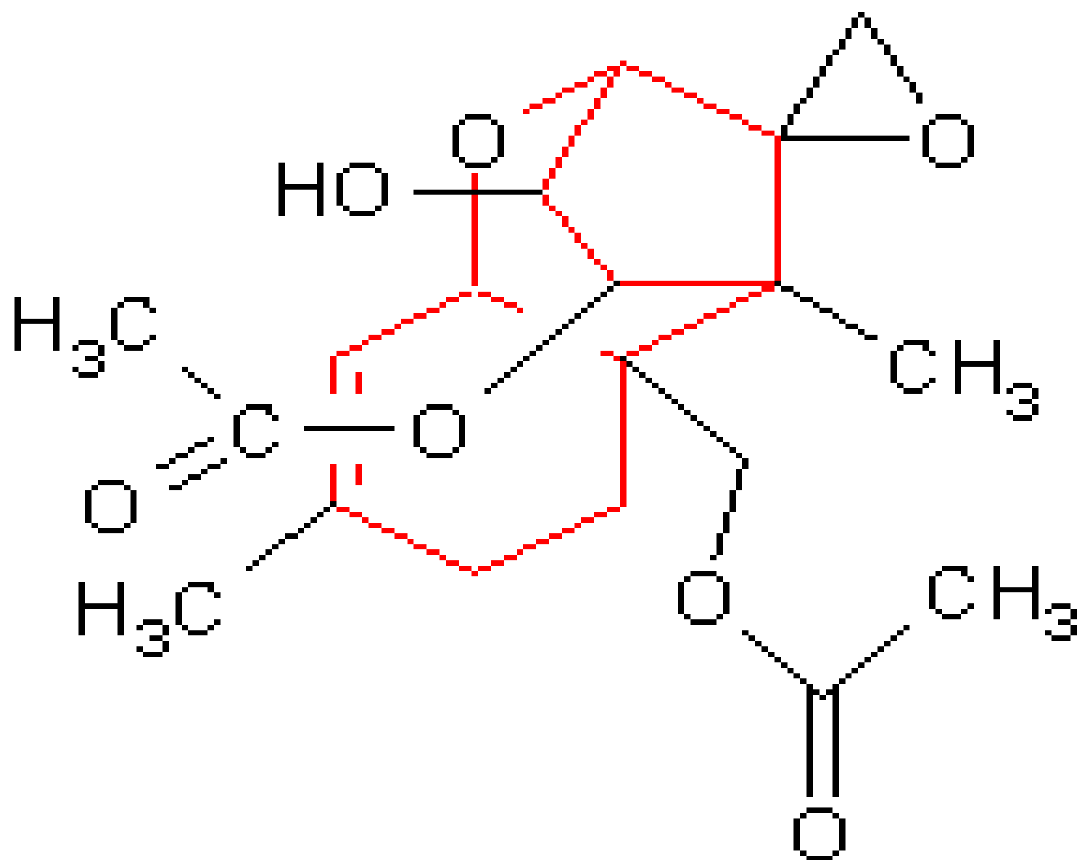


Fig 6 Structural formula of type T-2 Toxin

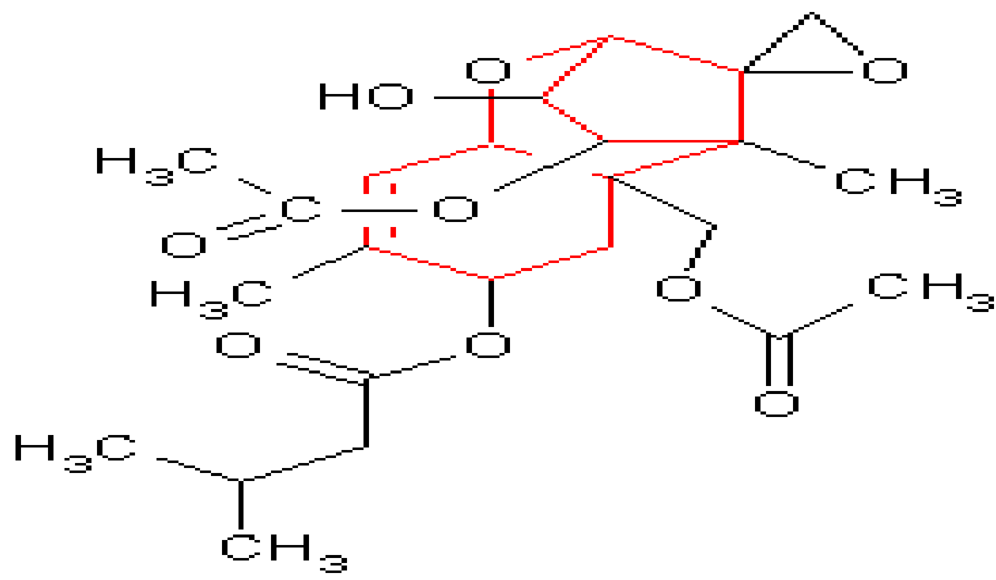


Fig 7 Structural formula of Diacetoxyscirpenol

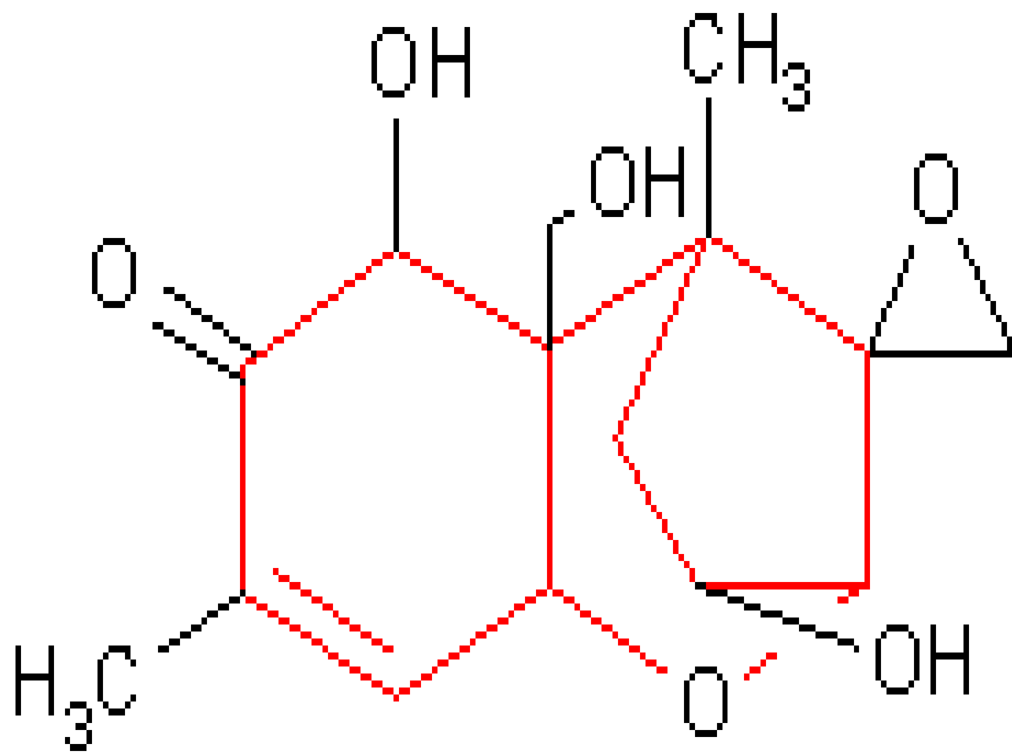


Fig 8 Strucrual formula of Deoxynivalenol (VOMITOXIN)

If mycotoxins had only affected animal productivity it is doubtful if they would have aroused much interest. Trichothecenes are known to be immunosuppressive compound and to inhibit protein synthesis (Prelusky et al., 1994; Cheeke 1998). They may cause neural disturbance, haemorrhages, skin irritation, vomiting, and diarrhoea and reduced feed intake (Kuiper-Goodman 1994; Cheeke 1998). T-2 has been reported to be the cause of alimentary toxic aleukia in humans in Russia and DON was the cause of human toxicosis in Japan, China and India (Beardall and Miller, 1994).

Deoxynivalenol has been indicted as a potent feed inhibitor in pigs. It is commonly called vomitoxin or DON (D'Mello and Macdonald1997).It also causes lymphopaenia in experimental animals (Clifford et al.,2003)

Diacetoxyscirpenol(DAS)and T-2 toxins have been implicated in field cases of mycotoxicosis in ruminants.T-2 toxin is over 400-fold more toxic than DON, and produce cytotoxic effects by inducing lipid peroxidation via the generation of free radicals (Atroshi et al., 2002;D'Mello , 2003;Rizzo et al. ,1994). T-2 toxin and DAS have also been reported in human leukaemia HL 60-cells (Nagase et al., 2001).

In general, trichothecenes of type A are more toxic than those of type B, and DON is the least toxic and T-2 the most toxic of the

commonly detected trichothecenes (Rotter et al., 1996; Scott 1990; Eriksen and Alexander 1998).

2.4.6 ZEARALENONE

Zearalenone (ZEN) may cause reproductive and infertility problems in animals as well as immunotoxic effect (Peska and Bondy ,1994;Prelusky et al., 1994). The presence of ZEN in feeds causes hyper - estrogenism,especially in swine, and has for long been a problem (Third Joint FAO/NAO/UNEP International conference on mycotoxins 1999;Hussein and Brassel,2001). In cows, infertility, reduced milk production and hyperestrogenism have been linked with ZEN or with fusarium species producing the mycotoxin (Mirocha et al .,1989). As little as 14mg ZEN/ kg is sufficient to cause infertility. When diary heifers were fed ZEN over three oestrous cycles, conception rate declined from 87% to 62% (Weaver et al., 1986). More recently, Towers and Sprosen (1993) implicated ZEN from pastures in New Zealand in the development of infertility in cows and sheep (D'Mello et al., 1997). In Puerto Rico ZEN has been considered as a possible agent that caused pubertal changes among young children (Saenz de Rodriguez 1984). Estrogenic activity of ZEN related metabolic have also been reported (Rizzo et al.,1994; Kuiper-Goodman et al., 1987; Kennedy et al., 1998).

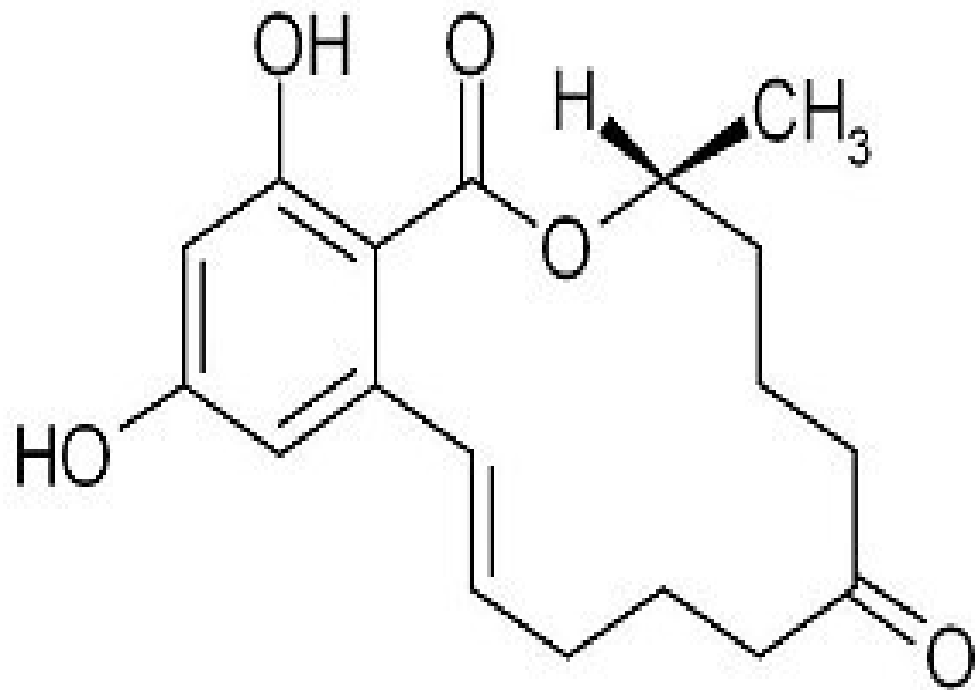


Fig 9 Structural formula of zearalenone

2.4.7 STABILITY OF MYCOTOXINS

Mycotoxins are relatively heat stable within the range of conventional food processing (European Commission 1994). Major losses of DON and other fusarium toxins were not observed during clearing, milking and baking processes (Scott et al., 1984). Operationally the persistence of trichotheces and mycotoxins generally makes them a threat even to military forces with protective equipment (Fang ,1983). Soviet troops in Afghanistan avoided operating in areas where these toxins were used (Wannemacher and Wiener, 1997). The toxins are stable in air and light for weeks and can withstand heat; a temperature of about 260^{0}C is required to destroy T-2 (Trusal ,1985; Wannemecher and Wiener ,1997).The concentrations of DON, 3-Ac DON and ZEN have been shown to increase during malt germination and DON was found to migrate to beer (Schwarz et al., 1995).Scott (1990) summarized the effect of food processing on trichothecenes and concluded that carry over of DON into processed cereal food may be considerable.

2.4.8 FACTORS AFFECTING PRODUCTION OF MYCOTOXINS

Moisture content and temperature are said to be the most critical factors affecting mould growth, but several factors are also important, such as spore inoculation, mechanical injury, insect damage, storm and rainfall damage to crops, plant stress, time mineral introduction of the plant,

chemical treatment, rapidly of drying, rewetting from condensation in storage, leakage in storage and hot spots (Hesseltine ,1976; Birzele et al., 2000). The hot spot is a small area with only a few kernels which are moist and may start the mould growth in a lot (Hesseltine ,1976). However, an interaction of several factors operating simultaneously is usually more important than a single factor in mycotoxin production (Moss ,1991). For example, studies with *Fusarium moniliforme* suggest that both time and water activity can interact to influence fumonisin B_1 production in maize kernels, while temperature may be important in the production of ZEN in other Fusarium species (D'Mello ,1997) of particular relevance in tropical region is the effect of insect invasion of seeds in the field storage. It is now agreed that insect infestation may predispose grain to fungal infection and therefore to mycotoxin production (Farrar and Davis ,1991). It is essential to realize that mould growth on the grain does not necessary mean that they are contaminated with mycotoxins and vice versa (Fink-Gremmel ,1999). Mould growth may not be visible on the surface of the kernel due to drying or the use of fungicides although even high amounts of mycotoxin may be found. Social conditions and behaviours such as methods of preservation, traditional feeding and environmental pollution may play a significant role in growth of mould and mycotoxin production (Ma'aroufi et al.,1996).

2.4.9 MECHANISMS OF ACTION

The many mechanisms by which trichothecenes produce toxicity are varied and their relative importance in illness is not fully understood (Coulombe ,1993). These however include:

a. Inhibition of protein synthesis. This is thought to be the most important effect (Ueno et al. ,1984;Chan and Gentry, 1984)

b. Inhibition of DNA synthesis (Agrelo and Schoental ,1980; Thomson and Wannemacher 1986)

c. Impairment of ribosome function (Forsek and Peska ,1985;Coulombe,1993)

d. Inhibition of mitochondrial protein synthesis (Tolleson et al., 1996, Trusal ,1985)

e. Induction of irreparable single cell breaks in DNA (Rizzo et al., 1998)

f. Immunosuppression allowing secondary and opportunistic bacterial infections and possibly delayed hypersensitivity (Jagadeesan et al., 1982;Prelusky et al., 1994; Cheeke 1998)

g. They may cause neural disturbance haemorrhage, skin irritation, vomiting diarrhoea and reduced feed intake (Kuiper-Goodman 1984; Cheeke 1998).

Trichothecenes react readily with Thiol group and at low concentration, inhibit thiol enzymes (e.g. creatine Kinase, Lactate dehydrogenase) (Ueno et al., 1984). They can be incorporated into lipid or protein elements of cell membranes. Tissue culture studies show alterations of membrane function (Pfeiffer et al., 1988). Sulfhydryl effects in cell membranes are important in cell to cell interaction, in the immune system. T-2 toxin induces cell membrane injury with haemolysis, apparently via a free radical mechanism.

Metabolism may be more important in detoxification than in producing toxicity. Unlike the aflatoxins that require metabolic activation, the trichothecenes are only toxic without activation as their prompt effects on the gastrointestinal mucosa with epithelial cell necrosis suggest. T-2 and other trichothecenes are deacetylated in the liver (Canady et al., 2001).Metabolites are also toxic but less so that T-2 (Ueno et al .,1984).Carboxyesterases (- SH serine esterases) in liver microsomes hydrolyse T-2 to the less potent HT-2. These enzymes may be clinically important. Inhibition of these enzymes by paraoxon (an organophosphate pesticide) in subclinical doses increases the toxicity of

T-2 in mice (Johnsen et al., 1986). Other potent inhibitors of the enzyme are tri o-cresyl phosphate (TOCP, an organophosphate), serine (a carbamate), and diisopropyl fluorophosphates (DFP a weak organophosphate nerve agent). These all inhibit hydrolysis of T-2 (Johnsen et al., 1986). This raises the possibility that similar compounds, such as PB; low level of nerve agent; or other carbamates or organophosphate insecticide might enhance the toxicity of T-2 or other trichothecenes at low levels.

2.5 MORPHOLOGY AND FUNCTIONAL EFFECTS OF MYCOTOXINS OF FUSARIUM OXYSPORUM

2.5.1 CYTOLOGICAL ALTERATION

a. NUCLEUS

One of the most prominent effects of mycotoxins on the nuclear structure is the alteration of hepatocelullar nucleolus .This initiates a decrease in nuclear components accompanied by a gradual redistribution of nucleolar component and appearance of dense microsphere. In the rat hepatocytes, micro segregation is induced by sub lethal doses of aflatoxinB_1 (Kenji and Mikio, 1978)

Pong and Wogan(1974) have investigated the time course effect or rate of 1mg/Kg of aflatoxin B_1 in both RNA polymerase activity and

hepatocytonucleolar ultra structure. With 1mg of dosing with afltoxin B_1, nucleular macro segregation was observed. This effect was still present 12hours after dosing but 36 hours later nucleolar macro segregation was not demonstratable and the fibrillar and granular components were well integrated. Correlating with the morphological changes of the nucleolus. Both Mg^{2+}activated and $Mn^{2+}(NH_4)_2SO_4$ activated RNA polymerase activity, were maximally inhibited by about 60% 15minutes after dosing. This inhibition continued to 12 hours. By 36 hours, enzyme activity had returned to pre-treatment levels.

b. MITOTIC ABERRATION

Mitotic aberrations caused by mycotoxin in vivo and in vitro have been reported. These pathological changes have been compared with nuclear effects of coleticine and thioacetamide. This is marked by mitotic stimulation in the liver and abnormal mitosis in the experimental animal (Kenji and Mikio, 1978).DAS produces similar chromosomal abnormality when L cells are exposed to mycotoxins (Kenji and Mikio, 1978)

Chromosomal abnormality have been produced by afltoxinB_1 in plants, animal and human cell cultures (Kenji and Mikio, 1978).The chromosomal aberration of aflatoxinB_1 include gap breaks ,fragmentation, deletion and translocation.

Mitochondria

Mitochondria aberrations are encountered after administration of severe mycotoxins. They are characterised by irregular profiles, swellings, and blob like evaginations(Enomoto and Ueno ,1974; Kenji and Mikio,1971)

Mitochondrial damage was put forward early as one of the initial causes of liver damage by CCl_4 intoxication. Two possible mechanisms are conceivable

-Damage of mitochondrial membrane

-Calcium accumulation

On damage to mitochondrial membrane, Christie et al (Kenji and Mikio, 1978) proposed that CCl_4 or its metabolite may attack the membrane structure directly or through peroxidation reaction. DAS is known to produce cytotoxic effects by induction of lipid peroxidation and generation of free radicals (Rizzo et al .,1994).In the case of inteoskyrin, Ueno(1974) found that it bound preferentially with mitochondrial membrane (Kenji and Mikio, 1978).

For the second possibility, evidence indicate that mitochondria in vivo and in vitro takes up enormous amount of Calcium which exist as Calcium phosphate, eventually destroying the mitochondria through

-Destruction of energy conservation mechanism of oxidative phosphorilation.

-Loss of intermitochondrial components, such as nucleotides and metal ions

-Irreversible changes in the m

2.5.2 HISTOPATHOLOGICAL DAMAGE

a. LIVER

Many mycotoxins affect the liver since it is the central organ of chemical metabolism, and is the organ which receives topographically concentrated mycotoxins after ingestion (Enomoto and Saito , 1972).The most consistent histopathological finding of liver injuries after administration of various mycotoxins are hepatocellular necrosis, haemorrhage and fat infiltration. Levels of assault of 1/4-1/2 of L_D50 usually result in massive cell necrosis, whereas 1/10 or less of the L_D50 results in single cell necrosis (Kenji and Mikio, 1978)

Fatty infiltration in the liver is the most pronounced and consistent pathological feature when toxic exogenous chemical substances are ingested by animals. In severely affected animals, the change is diffuse but more severe around portal areas (Kenji and Mikio, 1978).

b. KIDNEY

The kidney is frequently affected by various mycotoxins. Macroscopically the affected kidney are always enlarged and pale.Nephrotoxic mycotoxins can be divided into two groups depending on target tissues.

First group include mycotoxins which act preferentially on the nephron

Second group comprise those acting on the tubules.

The clinical signs of intoxication induced by the later are not prominent and consistent, and spreads to the mucous membrane and sub mucous layer of the ureter and urinary bladder (Kenji and Mikio, 1978; Uraguchi,1971). AflatoxinB_1,AflatoxinC_1,strerigmatocystin and cyclopeazonic acid show typical pathological changes of the nephron marked by enlarged nuclei containing marginal chromatin and necrosis are often seen in the proximal and or distal tubules in the rat and sheep. In contrast with the urinary tubule, the glumerulus is affected only slightly (Uraguchi, 1971).

CHAPTER THREE

3.1. MATERIALS AND METHODS

3.2.0 CULTURE PREPARATION

An isolate of *Fusarium oxysporum* was obtained from the mycology laboratory of the department of crop protection A B U Zaria. It was subcultured in the same laboratory in Petri dishes containing potato dextrose agar culture media .The cultures were left for four weeks in an incubator to attain full growth at 24^0C

3.2.1 MATERIALS

Wire loop, flame, culture samples of *Fusarium oxysporum,* potato dextrose agar culture media Petri dishes, distilled water, 1litre volumetric flask, autoclave, sorghum.

3.2.2 PREPARATION OF CONTAMINATED GRAINS

The sorghum grains were autoclaved in the preparatory room of the crop protection laboratory. The kernels were then inoculated with the *Fusarium* isolates and allowed to culture (200g of sorghum and 120ml distilled water) in a one litre volumetric flask. This is because *Fusarium* isolates are known to produce high levels of mycotoxins when grown this way (Abbas and Mirocha ,1984)

The culture were incubated at 24^{0}C for two weeks until full growth was observed

The grains were then dried and broken to a small consistency to be compounded into feeds

3.3. FEED PREPARATION

The inoculated grains were then mixed in various ratios with commercial rat feed and compounded into feeds

One part contaminated grains three parts commercial rat feed[group2]ie 25% contaminated feed

Two parts contaminated grains two parts commercial rat feed[group3] ie 50% contaminated feed

Three parts contaminated grains one part commercial rat feed[group4] ie 75% contaminated feed

Whole contaminated grains[group5] ie 100% contaminated feed

The feeds according to their groupings were moulded into pellet forms using starch as a binder and then dried.

3.3.1 ANIMAL PREPARATION

Thirty animals of the wistar rat family were obtained from the animal house of the faculty of pharmaceutical science A.B.U.Zaria. The animals were sexed and randomly sorted into five groups of six and housed in separate cages for each sex. They were allowed for two weeks to acclimatize before the experiment will commence.

3.4. FEEDING REGIMEN

The animals were provided with feed and water ad libitum in their cages and according to their groupings

Group one-------------------------------------control

Group two-------------------------------------25%contaminated feeds

Group three------------------------------------50%contaminated feeds

Group four-------------------------------------75%contaminated feeds

Group five-------------------------------------100%contaminated feeds

3.5. HEMATOLOGY

Blood samples were collected from each animal before sacrifice from the tail region. Haematological analysis were done according to the method of Schalm et al., [1975].

3.6. CALCULATIONS

The Improved Neubauer haematocytometer was used both for red blood[RBC]cells and white blood[WBC] counts

For the differential WBC count ,the Leishman staining technique was employed

Calculations were done to arrive at the results

$$W BC = \frac{W \times DF \times 10^3}{A X D}$$

W= Total number of leucocytes counted (per L)

DF = Diluting factors (20)

A=Area of chamber counted ($5mm^2$)

D=Depth of chamber counted ($0.1mm^2$)

$$RBC = R = \frac{N \times DF \times 10^6}{A X D}$$

Where R = Red cells counted (per L)

N = Number of cells counted

DF = Diluting factor (200)

A = Area of chamber (0. 2mm^2)

D = Depth of chamber (0.1mm^2)

3.7. EXPERIMENTAL PROCEDURE

The animals were housed in hygienic plastic cages and maintained under standardized condition of light (12 hour's light/dark cycle) with fresh tap water. After twenty eight days (28) the animals were sacrificed by cervical dislocation(in order to observe pathological changes and any possible regenerations or recovery) and their livers and kidney organs removed and immediately fixed in 10% neutral buffered formalin.

3.7.1 LABORATORY MATERIALS

Cages and water bottles

Dissecting kit

Weight balance

Automatic tissue processor

Fume box and cotton wool

Microtome (Leitz welter model)

Water bath

Glass and cover slips

Light microscope

Photo microgram

Digital microscopic camera

3.7.2 CHEMICALS

Ethanol

Formalin

Halothane

Xylene

Paraffin wax

DPX mountant

Haemotoxylin and eosin stains

Distilled wate

3.8. METHODOLOGY

The rats were weighed and then euthanized with cotton wool soaked in fluothane (halothane) in a fume cup board for five minutes. They were then dissected and livers and kidney removed.They were

observed for any gross lesion or changes before they were fixed in 10% formalin and prepared for tissue analysis.

For tissue analysis, they were washed thoroughly in water to remove excess fixative. This was followed by passing the tissue through graded concentrations of ethanol in an ascending order from 70%, to 95%, 99% and absolute (100%) to dehydrate the tissue. The tissue processing lasted twenty four hours (24hours) in the automated machine .The tissues were then cleared in two changes of xylene to remove excess ethanol for a time period of one hour (1hr) each .The tissues were then infiltrated with molten paraffin wax (embedding media) in a plastic embedding mould in an oven set at 60^{0}C for one hour ,this is to allow solidification, in order to obtain a firm homogenous mass containing the embedded tissue.

Sectioning was done using a rotatory microtome (Leitz Wetzlar).Sections were about 10µ (microns).Sections were floated in a warm bath, then transferred to clean slides smeared with albumen in order to stick them firmly and to pick stain well .The glass slides were put in an incubator at 37^{0}C for 30minutes.This is to allow for evaporation of water and for sections to get attached to the glass slide. Tissues were then passed through two changes of xylene for five minutes (5min) each to dewax the sections then through decreasing ethanol concentrations (100%, 95%,

and 70%) for five minutes each to remove excess xylene. Sections were then passed under a running tap to remove excess xylene. They were then stained using haematoxylin and eosin stain, before being passed under running water to remove excess stain .Again they were passed through graded concentration of ethanol (70%, 95%and 100%) and cleared with xylene. Mounting was done using DPX mountant, the mounting medium. The cover slip was applied while slides were allowed to dry. Micrographs were then taken using Canon powershot A80 Camera.

CHAPTER FOUR

4.1. RESULTS

4.2. HEMATOLOGICAL INDICES

The results of the study of the effect of oral ingestion of *Fusarium oxysporum* inoculated grains on the haematological indices of the wistar rat are represented in the table below.

Table 1. The effect of dietary Fusarium oxysporum on the red blood cell count.

Blood cells	Control Grp 1	25%Cont. Grp 2	50%Conta. Grp 3	75%Conta. Grp 4	100%Conta. Grp 5	F.	P.
RBC (10^{12}/L)	4.210 ± 0.834	4.340± 0.873	4.590± 0.479	4.340± 0.873	5.040± 0.790	1.061	0.396

Table2. The effect of dietary Fusarium oxysporum on the white blood cell count.

Blood cells	Control Grp 1	25%Cont. Grp 2	50%Conta. Grp 3	75%Conta. Grp 4	100%Conta. Grp 5	F.	P.
WBC (10^9/L)	6.063 ± 1.559	5.225± 1.701	5.142± 0.753	5.225± 1.701	7.033± 1.305	1.906	0.141

Table3. The effect of dietary Fusarium oxysporum on the neutrophil,lymphocyte,monocyte,basophil and eosinophil cell count.

Blood cells	Control Grp 1	25%Conta.Grp 2	50%Conta. Grp 3	75%Conta. Grp 4	100%Conta. Grp 5	F.	P.
Neutr (%)	25.667±5.922	25.167±4.070	27.833±14.049	25.167±4.070	29.667±3.502	0.428	0.787
Mono. (%)	3.333±1.211	3.833±1.169	4.167±1.329	3.833±1.169	3.000±1.265	0.848	0.508
Lymph. (%)	67.667±6.802	66.333±3.3886	65.500±15.228	66.333±3.386	64.000±4.336	0.170	0.952
Eosino. (%)	2.833±0.753	4.000±0.894	2.500±1.871	4.000±0.894	3.000±0.894	2.229	0.095
Basoph(%).	1.000±0.000	1.000±0.000	0.000±0.000	1.000±0.000	1.000±0.000	1.000	1.000

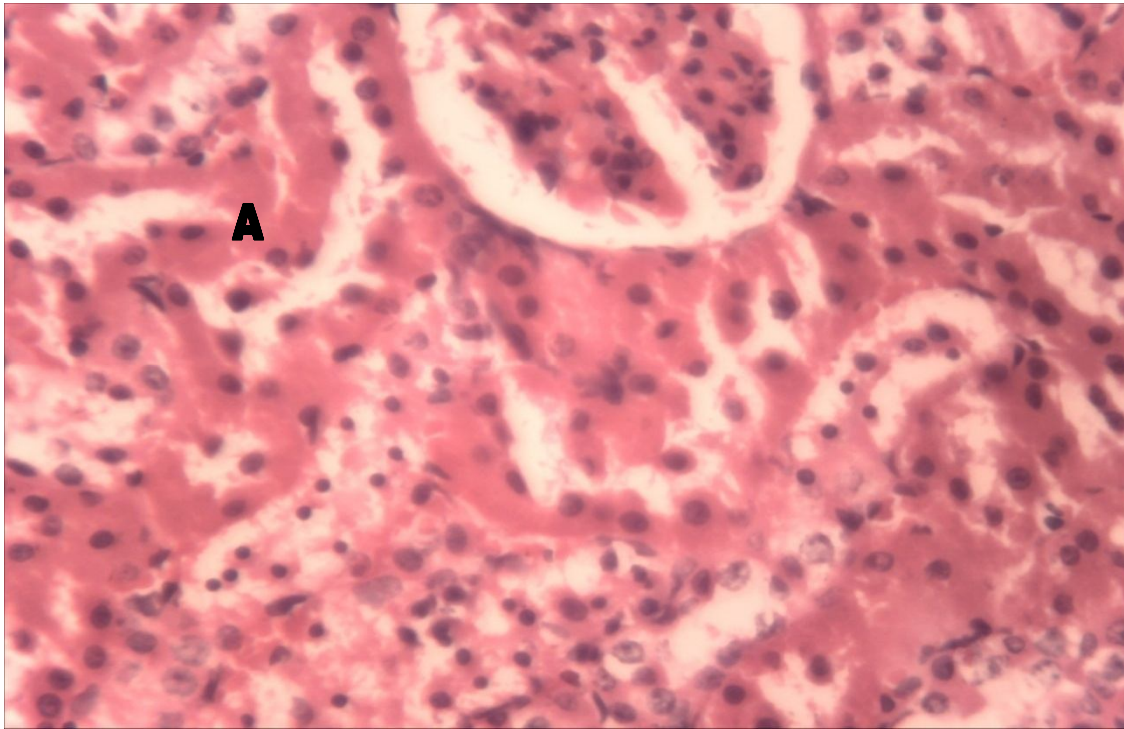


Plate 1; transverse section of kidney

H/E Stain; control group; Mag x250.

The nuclei and cytoplasm are normal staining,the proximal convoluted tubule(A) is also normal.

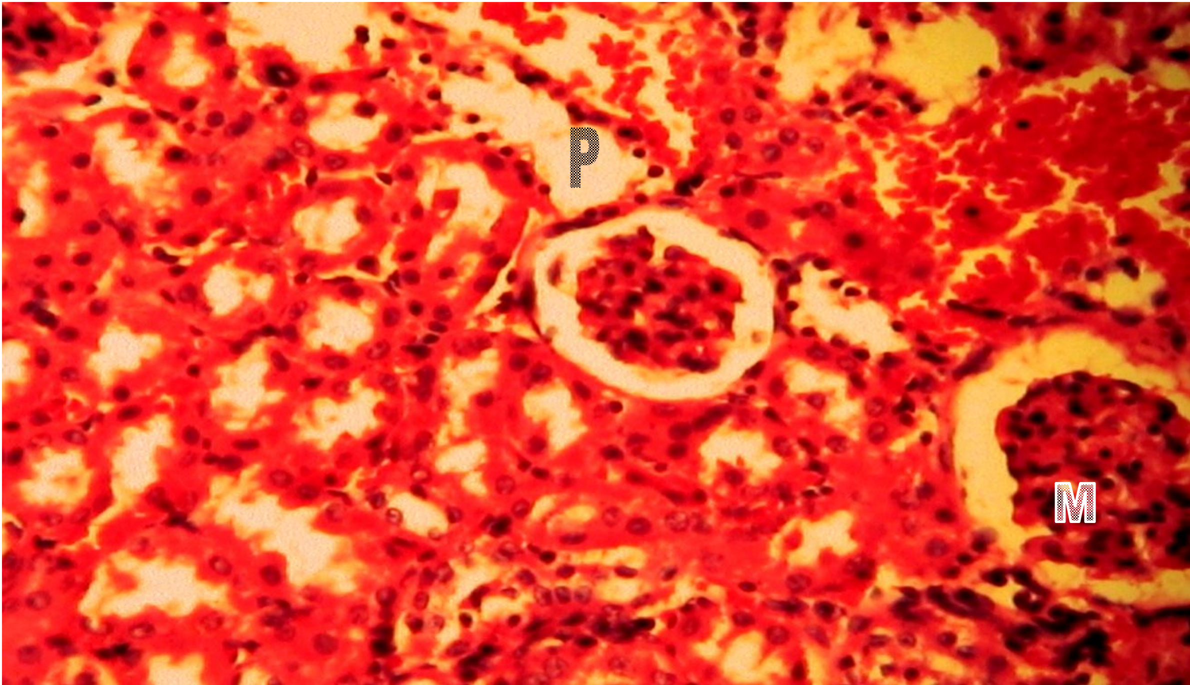
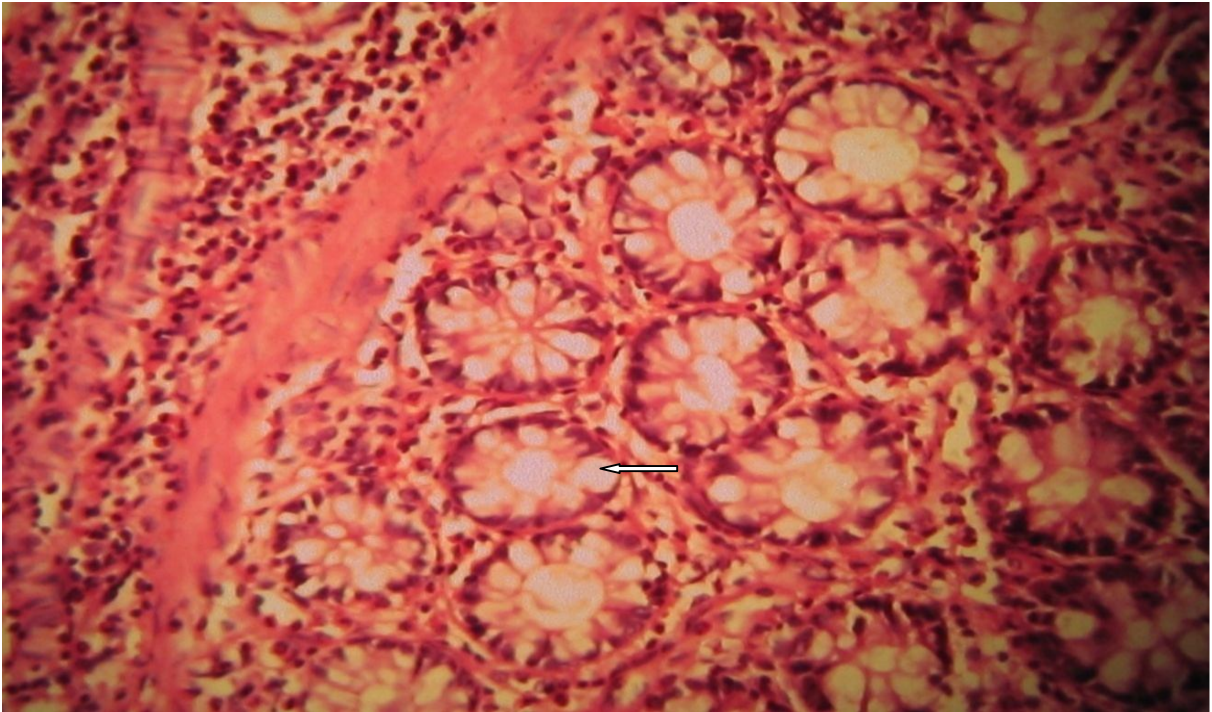


Plate 2; Transverse Section of kidney

H/E Stain; experimental group 2; Mag x250

At 1:3 part rat feed: *Fusarium oxysporum* inoculated grains; the proximal convoluted tubules(p) has degenerated. Notice the infiltration of mononuclear cells(M) in the glomeruli.



H/E Stain; experimental group 3; Mag x250

Plate 3; Transverse section of the kidney

Photomicrograph of the renal cortex of a rat fed 1:1part rat feed: *Fusarium oxysporum* inoculated diet.The tubules have typical cloudy swellings (white arrow) in the cytoplasm.

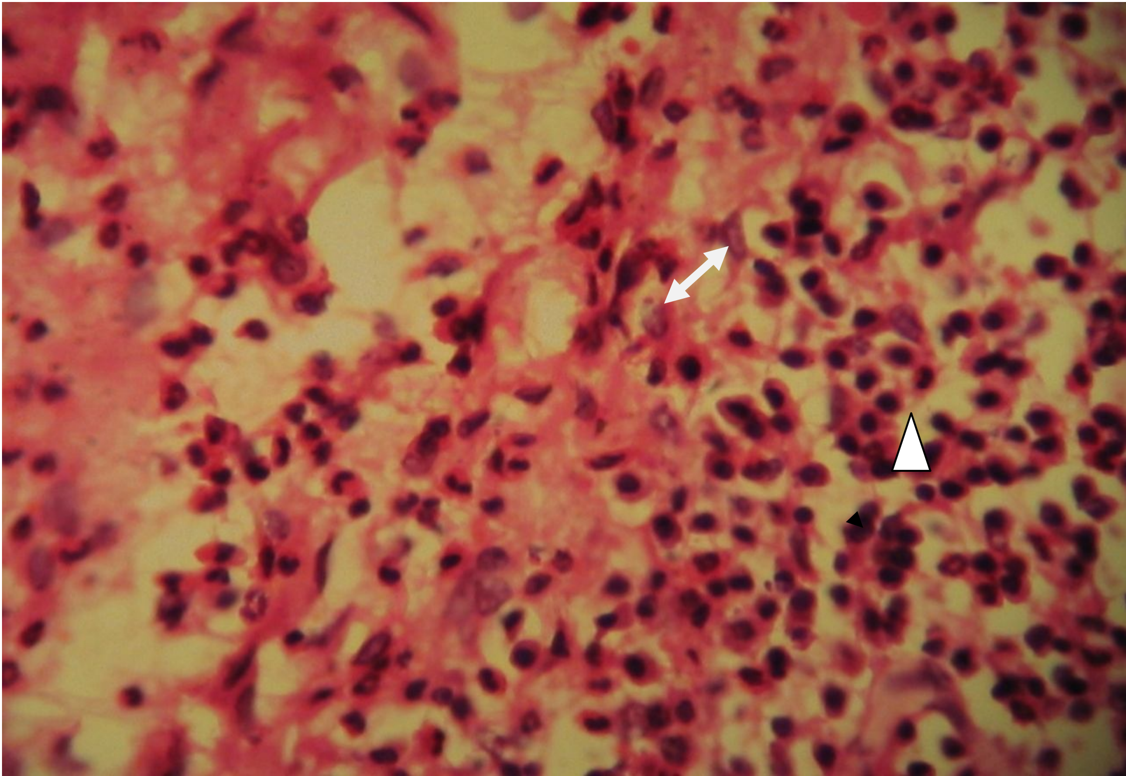


Plate 4; Transverse Section of the kidney

H/E Stain; experimental group 4; Mag x250

Photomicrograph of the renal cortex of a rat fed 1:3part rat feed: *Fusarium oxysporum* inoculated diet. Notice apoptotic cells (white double arrow) mononuclear cells invade the interstitium(arrow head).

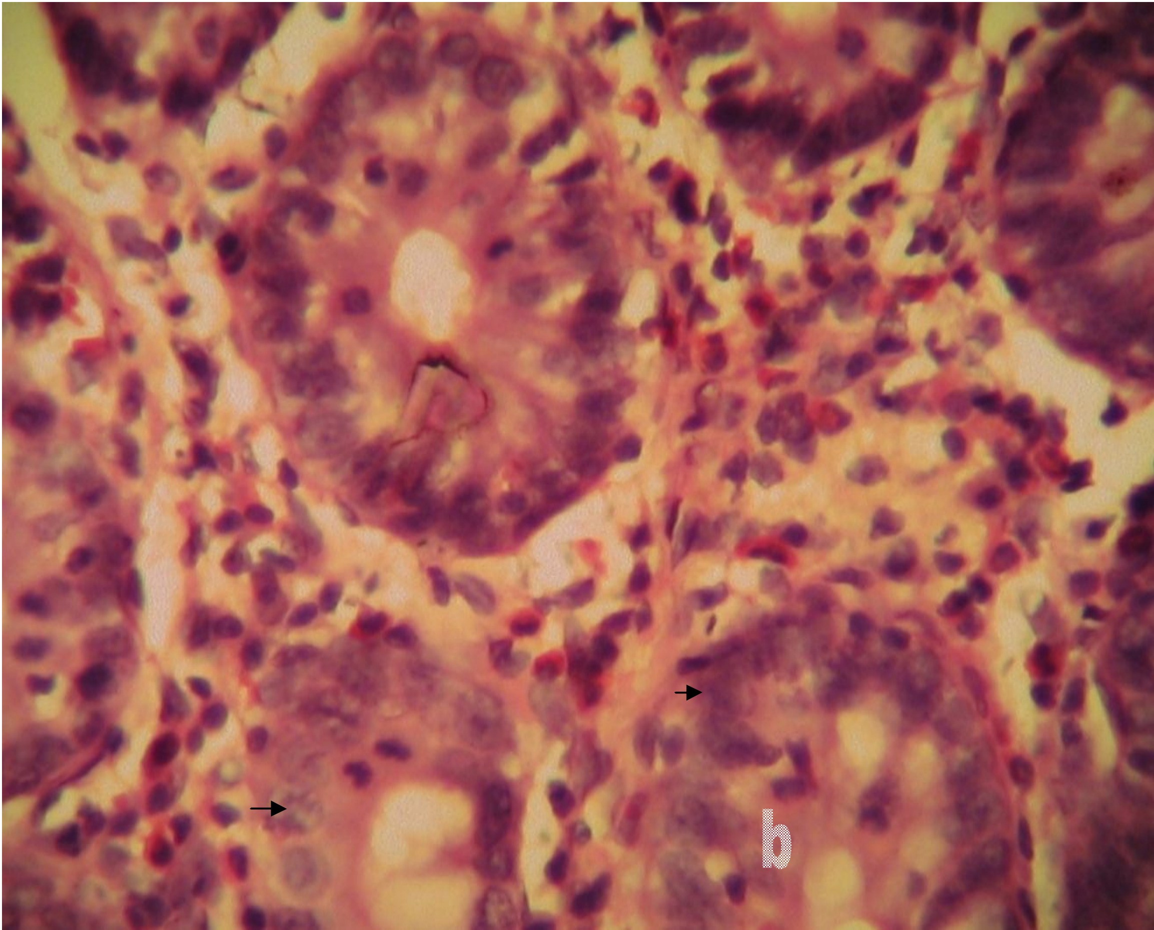
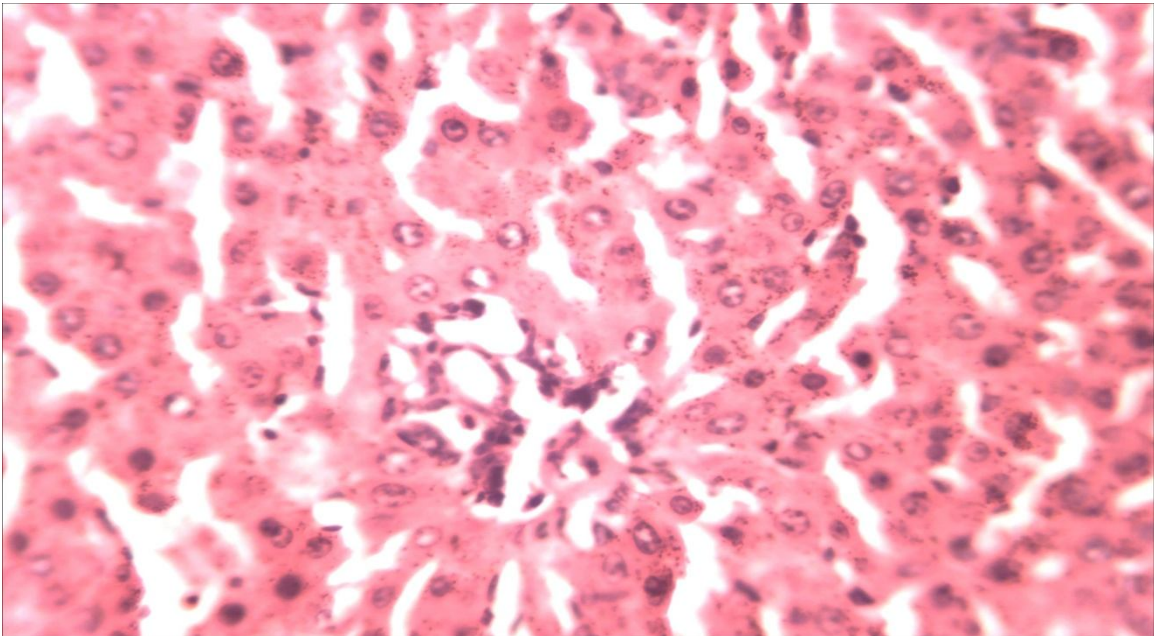


Plate5; Transverse Section of the kidney

H/E Stain; experimental group 5; Mag x 400.

Photomicrograph of renal cortex of rat fed whole inoculated grains.Notice the widespread karyorrhexis(arrow).Dissolution of the cellular structure and loss of cytoplasmic content(b) are seen around the nuclear fragment of karyohexic cells.



H/E Stain; Control group; Mag x250.

Plate 6; Transverse Section of the Liver

Photomicrograph of rat from control group.

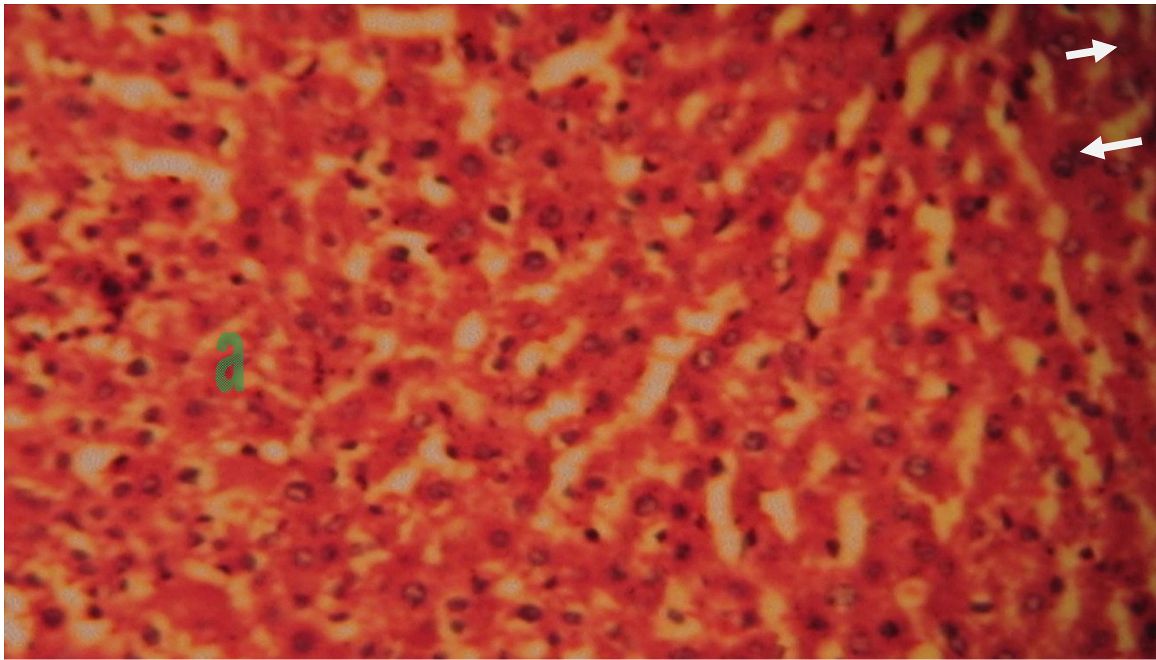


Plate 7; Transverse Section of the liver

H/E Stain; experimental group2; Mag x250

Cellular inflammation can be observed at the background with sparse infiltration of mononuclear cells(arrow).Fragments of cellular remains are seen(a)here and around.

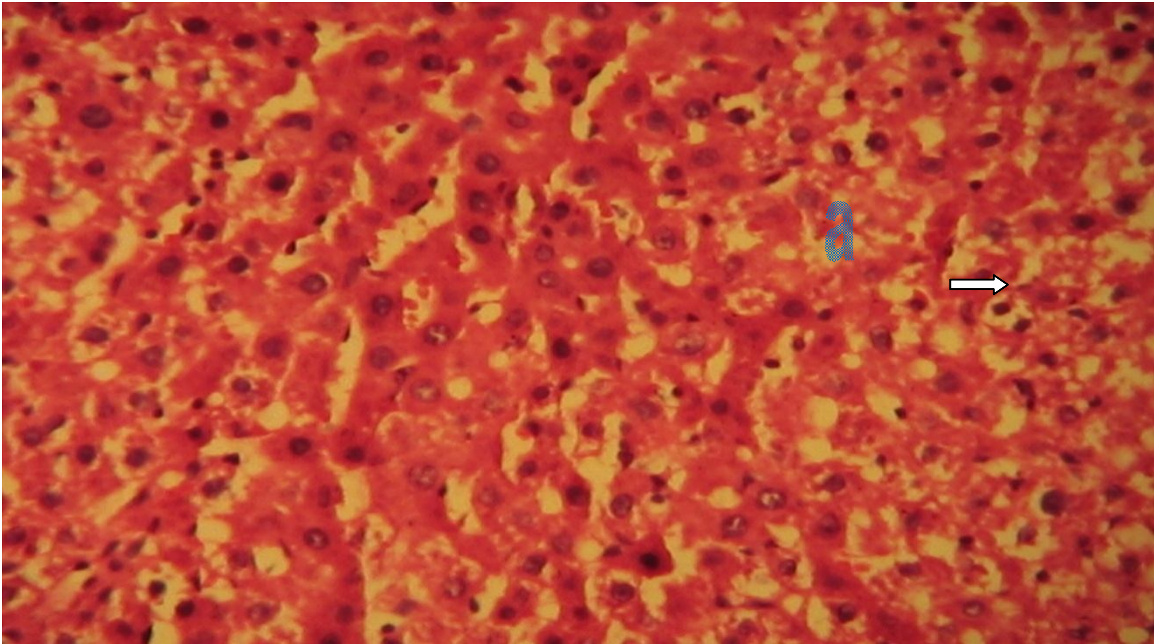


Plate 8; Transverse Section of the Liver

H/E Stain; experimental group 3; Mag x250

Inflammation has increased with possible exudation(a).Cellular debris are probable remains of dead cells increased in the hepatocytes.

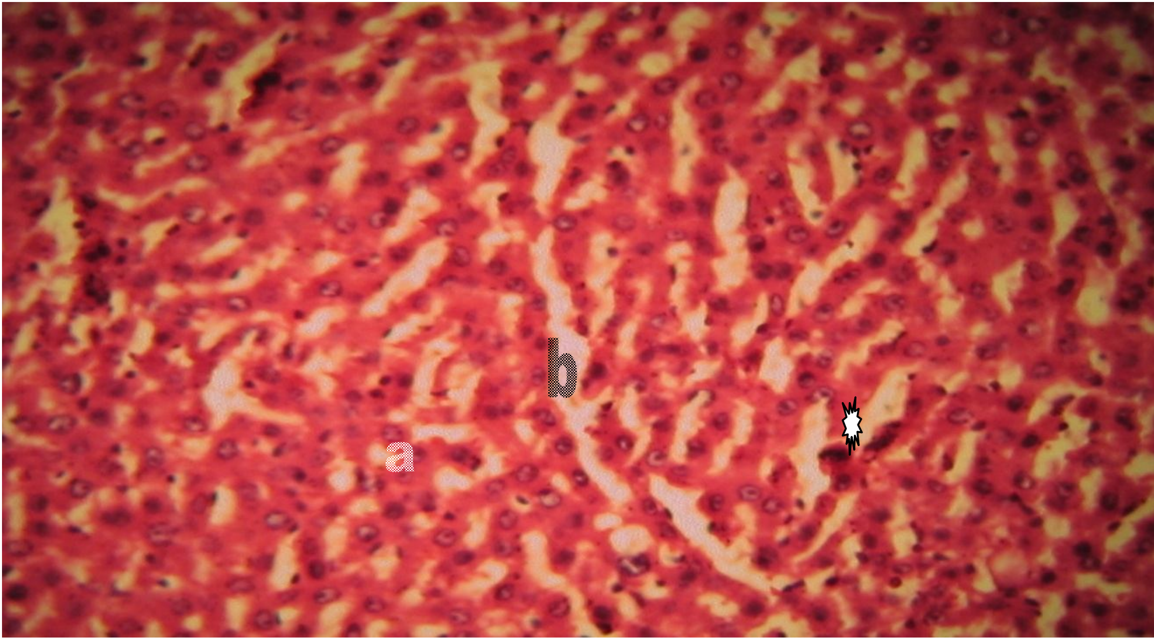


Plate 9;Transverse Section of the Liver

H/E Stain experimental group 4;Mag x250

Notice the pkynotic nuclei in a dense eosinophilic

Cytoplasm(asterisk).Widened sinusoidal spaces(b) are also prominent.Cellular debris are even more abundant.

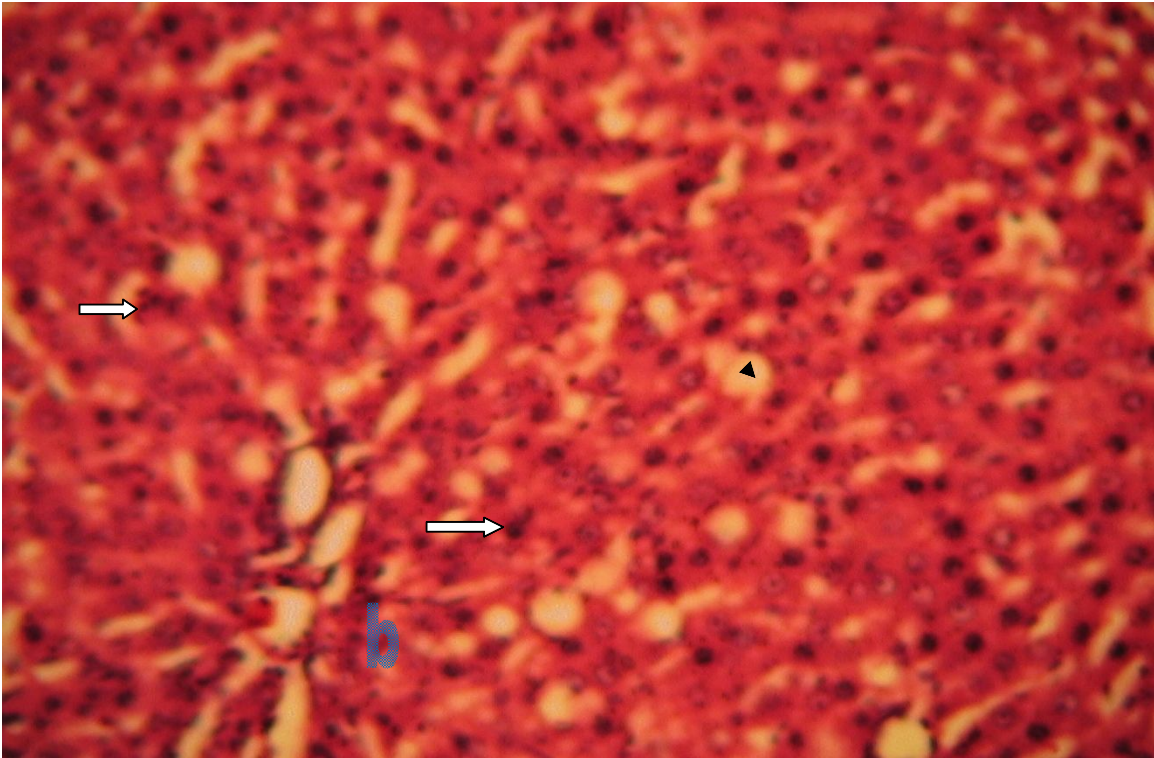


Plate 10; Transverse Section of the Liver

H/E Stain; experimental group 5; Mag x250

Apoptotic bodies are few in this photomicrograph(arrow).Cellular debris abound here(b) and elsewhere.The architecture of the hepatocytes has been destroyed around this portal triad.

4.3 KIDNEY PATHOLOGY

In the kidney of the experimental group, there was marked cytoplasmic eosinophilia and the disappearance of cellular contours. Necrotic cells show an increase in eosinophilia, which is attributed in part to the binding of eosin to denatured intracytoplasmic proteins (Cotran, 1999). In lethally injured cells, catalytic enzymes derived from the lysosomes of either the dying cells or immigrant leucocytes start digesting the cytoplasm content, giving the cytoplasm a typical moth-eaten appearance(Cotran , 1999).This degradation of the cell progresses with increasing assault on the cell, with evident changes in the nucleus. The nucleus of dying cells become shrunken and condensed(pyknotic).With time, the chromatin of pyknotic cells break up into many clumps(karyorrhexis).Eventually the nuclei disappear(Cotran ,1999).

The cells in plate 3 have the typical cellular swelling .This is the first manifestation of almost all forms of cellular injury, and results from a shift of extracellular water into the cell. In toxic situation this is due to impairment of the sodium pump mechanism at the cell membrane. If water continues to accumulate within cells, small clear vacuoles appear within the cytoplasm(Cotran ,1999).The Bowman's space of the kidney is widened due to the coagulative necrosis within the Bowman's capsule.

The loss of cellular outline and nuclei seen in the renal tubule of the rats in the experimental group, suggest that *Fusarium oxyporum* induces tubular necrosis in the kidney. Following cell death, cell components are progressively degraded, and debris occur (Ozbek and Ozbek , 2003).Areas of debris, which contained mononuclear leucocytes, were observed in the kidney of the different experimental groups. The mononuclear cell infiltrations, indicating tissue inflammation, (Ozbek and Ozbek, 2003) were interpreted as a response to *Fusarium oxyporum* induced kidney damage in the experimental groups. It has been reported that DON and T-2 toxin induce lipid peroxidation by generating free radicals, causing disturbances in the structure of cellular membranes, and reduces the cellular level of glutathione by binding to a sulfhdryl group(Rizzo et al.,1994;Atroshi et al.;1997;Rizzo et al., 1998).These biochemical changes may be responsible for T-2 toxin induced cell injury(Rizzo et al.,1998).Nephritis, hepatitis and mucocutaneous eruptions have been reported in various animals with fusariotoxicoses,occurring as a result of consumption of feedstuff containing trichothecenes such as T-2 toxin.(Gabal et al., 1986)

Many apoptotic cells and bodies were observed in the kidney of the experimental group. Fumonisins has been demonstrated to produce the characteristic features of apoptosis in rat kidney including condensation of the nuclear chromatin, shrinkage of the cytoplasm, and formation of

apoptotic bodies that can be phagocytosed by macrophages(Tolleson et al.,1996).The trichothecenes DON has been shown to induce apoptosis in lymphoid organs in mice(Zou et al. ,2000),and apoptosis has been demonstrated in rat liver cells using agarose gel electrophoresis(Rizzo et al.,1998).Internucleosomal DNA fragmentation induced by trichothecene mycotoxins such as T-2 toxin and DAS has been reported in human leukaemia HL-60 cells(Nagase et al., 2001)

We encountered mitotic figures in group 2, the mildest experimental group.This may indicate an attempt to replace apoptotic and damaged cells.The loss of cytoplasmic content and attendant nuclear changes showed progression as we moved from group 3(moderate)to group5(very high dose).The karyyorrhexis is especially prominent in group5.

4.4 LIVER PATHOLOGY

Histological changes in the liver were examined by light microscopy.Hepatocytes from the experimental group showed marked necrosis.Necrotic cells had an increased eosinophilia that was attributed partly to increased binding of eosin to denatured intracytoplasmic proteins and condensing of nuclei (pyknotic nuclei). (Toner et al., ,1996;Cotran , 1999)

Dense eosinophilic hepatocytes with pyknotic nuclei seen in the liver of rats fed *Fusarium Oxyporum* diet suggest that *Fusarium Oxyporum*

induces hepatic cell death.Mononuclear cell infiltrations,indicative of tissue damage,are seen readily in the experimental groups.This has been interpreted as a response to fungus-induced liver damage in the experitmental groups. Apoptotic cells may be observerd.Tolleson et.al., (Tolleson et al.,1996)noted that the characteristic features of apoptotic cells are the condensation of the chromatin to the periphery of the nucleus,shrinkage of the cytoplasm and formation of apoptotic bodies,which can be phagocytosed by macrophages

Zhou et al.,Fawcett et al., (Zhou et al.,2000;Fawcett et al.,2000) reported that DON induces apoptosis in lymphoid organs.Leakage of cytochrome C into the cytosol,because of mitochondrial damage,can also trigger apoptosis as cytochrome C is an integral component of the electron transport chain(Cotran,1999).Trichothecenes mycotoxins such as T-2 toxin and DAS can activate caspase-9 activation in the human leukaemia HL-60 cells,and induce internucleosomal DNA fragmentation(Nagase,2001).Agarose gel electrophoresis demonstrated that T-2 toxin and DON caused 56% and 23% of hepatic DNA fragmentation,respectively compared with no toxins(Rizzo et al .,1998). Leal showed that in-vitro exposure of chicken hepatocytes to T-2 toxin resulted in cellular damage.

We observed persistant inflammation in the liver plates.This increased with attendant cellular debris in the plates.The liver tissue possibly did not recover from the acute assault,progressing thus into necrosis.Besides the necrotic hepatic tissue,widened sinusoidal spaces may be observed with destruction of hepatic architecture.This could possibly be due to loss of cytoplasmic tissue,consequent to necrosi

CHAPTER FIVE

5.1 DISCUSSION

5.2 HEAMATOLOGY

Analysis of both red blood and white blood cells did not show any statistically significant change in their values at the end of four weeks oral ingestion of Fusarium oxysporum inoculated diet. Although no literature was found to have reported any effects at the end of four weeks ingestion of *Fusarium* inoculated grains. Swamy and other workers reported that there was no significant difference, when pigs were fed Fusarium inoculated diets(Swamy et al.,2003; Accensi et al. ;2006;Swamy et al. ,2004).

When the differential white blood cell parameters were taken there was no significant difference found between the neutrophil of control and that of the experimental group. There was also no significant difference found between the lymphocytes values of the control and that of the experimental groups. The monocytes values between the control and the experimental groups also did not differ, there was no significant difference. There was however a significant difference between the eosinophil values for group two and group four ($P<0.05$).The other groups did not show any statistically significant response.

5.3 EFFECT OF FUSARIUM MYCOTOXINS ON TISSUE

The adverse effects of feeding diets contaminated with *Fusarium* mycotoxins, especially trichothecene mycotoxins to animals can be explained as the outcome of two events:direct and indirect effects (Swamy et al.,2004). The indirect effects include reduced food consumption, which will consequently lead to growth depression and increased susceptibility to diseases due to reduced availability of nutrients.

Direct effects of *Fusarium* mycotoxins on tissues and organs can provoke metabolic disturbances that are detrimental to the normal functioning of the animal. These can include the inhibition of tissue protein synthesis, altered nutrient absorption, cytotoxic effect on various cell types, altered apoptosis of lymphoid cells, oxidative damage, and cardiovascular toxicity (Bondy and Pestka, 2000).

When the pigs fed contaminated diets were compared with those fed control diet ad libitum, the differences observed could be due to both the direct and indirect effects of mycotoxins. This combined effect is the total effect. Direct or indirect effects can be equal to or less than the total effect. If either of these effects is greater than total effect, that

additional effect can be attributed to factors other than the effect of *Fusarium* mycotoxins. Chavez and Rheaume (1986) made a first attempt to differentiate the direct and indirect effect of *Fusarium* mycotoxins and concluded that the effect of DON on BW gain was indirect, due to a depression in feed acceptability and intake, but there seemed to be no direct detrimental effect on the measured blood variables.

Rotter et al,. (1994) reported lower skin temperature, better feed efficiency, more corrugated stomach, reduced -globulin levels, and lower antibody titers to SRBC in pigs consuming *Fusarium* mycotoxin-contaminated diet when compared with the pair-fed control pigs. The appearance of the stomach and antibody titers was not altered in pigs fed contaminated diet compared with pigs fed the control diet ad libitum; therefore, the above effects observed in the study of Rotter et al. (1994) , in comparison with pair-fed control pigs, do not represent a direct effect of mycotoxins. These effects, however, may be related to the stress of pair feeding. The effect on skin temperature, feed efficiency, and serum -globulin levels, however, was due to the direct effect of mycotoxins.

CHAPTER SIX

6.1 CONCLUSION

Based on the observations made on of the histological changes in the liver and kidney of wistar rats after oral ingestion of Fusarium oxysporum inoculated diets,it may be concluded that

- *Fusarium Oxyporum* causes marked inflammation and cellular damage to both rat kidney and liver.
- The severity of the damage caused by this toxins increases according to the dosage or concentration of contaminated grains ingested.

6.2 SUGGESTION FOR FURTHER WORK

Work needs to be done to determine the effect of *Fusarium oxporum* on other body tissues including the brain and uterus.

More work needs to be done to determine the effect of *Fusarium oxporum* over a long period of study.

More work needs to be done also to determine *Fusarium oxysporum* endemic areas to identify any correlations with liver and kidney disease

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