DIFFERENTIAL EFFECT OF ONE AND TWO WEEKS EXPOSURE TO CARBENDAZIM ON RENAL TOXICITY IN RAT

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN BIOCHEMISTRY.

JULY, 2021

CERTIFICATION

This is to certify that this project work was carried out by OGBEIDE WENDY OTITI under my supervision.

Prof. (Mrs.) M. A. Adaikpoh **(Supervisor)**	**Date**
Dr. S. I. Ojeaburu **(Project Coordinator)**	**Date**
Prof. (Mrs.) K. E. Imafidon **(Head of Department)**	**Date**
External Examiner	**Date**

DEDICATION

I would relish dedicating this report to The Almighty God, who has been my ultimate source of bliss, vigor, sapience, good health and sustenance for visually perceiving me through.

ACKNOWLEDGEMENT

Firstly, I want to thank my project supervisor, Prof. (Mrs.) M. A. Adaikpoh for her guidance, ideas, suggestions, corrections and efforts in making my project a success, I am indeed grateful.

And special thanks goes to my HOD Prof. (Mrs.) K. E. Imafidon and my lecturers for their immeasurable guidance in the course of this project work. I want to also appreciate my project coordinator, Dr S. I. Ojeaburu for his inspirational words of courage, thank you.

Also, special thanks go to my parents, Barr. and Mrs. Thompson Ogbeide for all their consistent advice, encouragement, support and prayers. I really appreciate my siblings for their support, and siblings ; Joyce Ogbeide, Winnie Ogbeide , Naomi Ogbeide and Mctom Ogbeide for their unending support and prayers.

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ABSTRACT

In this study, the differential effect of one and two weeks exposure to carbendazim on renal toxicity in rat was carried out. Twenty male rats were grouped into control and test group. The rats were labelled using picric acid and weight of individual rats were taken using a weighing balance and recorded as initial weight. The control group was orally administered 1% tween 80 using a gavage, while the test group was orally administered 200mg/kg body weight of Carbendazim dissolved in 1% tween 80 (acts as vehicle) using a gavage. oral administrations were done once and the rats were monitored for a period of 1-2 weeks and observations were recorded. After one week of Carbendazim exposure, five (5) rats from each group were anaesthesized in chamber containing Chloroform and blood was collected by cardiac puncture. The kidneys were excised, trimmed free of connective tissues and weighed. One kidney from each group was immersed in formalin and used for histopatholigical study while the remaining kidneys were stored at -4^0c until needed for biochemical studies. The parameters measured were; Superoxide Dismutase, Catalase ,Malondialdehyde ,Urea and Creatinine. There was no significant change ($p>0.05$) in kidney weight of rats when compared with the normal control but the weight of kidney decreased significantly ($p<0.05$). Increase in MDA levels were not significant ($p>0.05$)in carbendazim treated groups compared with normal control after 7days and 14 days respectively. The elevation in Catalse activity after 7 days was not significant($P>0.05$) in Carbendazim treated group when compared to the control but Catalase activity increased significantly ($P<0.05$)after 14 days which may be an indicator of the body trying to alleviate oxidative stress. increased Superoxide Dismutase (SOD) is beneficial in event of increased free radical production. SOD levels increased significantly ($p<0.05$)in carbendazim treated rats compared with the control after 7days of Carbendazim exposure. The significant increase in Urea and Creatinine levels in the blood may be attributed to Carbendazim toxicity. Creatinine levels increased significantly ($p<0.05$) after 7 days in carbendazim treated group compared with the normal control. After 14 days, the increase in Creatinine levels was not significant ($p>0.05$) in Carbendazim treated group compared with control. In histopathological examination, Ultra-structural changes were observed in the kidney of rats treated with carbendazim compared to those of control. Inflammatory infiltrates and tubular necrosis which indicates damage to kidney tubules were observed in the kidney of carbendazim treated rats (plate 2 and plate 4) after 7 days and 14days respectively.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Pesticides have been applied extensively in agricultural and veterinary practices worldwide. The global annual consumption of pesticides is approximately two million tonnes where Europe accounts for 45%,USA, accounts for 25% and other countries accounts for the remaining 25% usage. Fungicides account for 2% of the total annual consumption of pesticides in India (De *et al*., 2014).

Carbendazin (methy 1H – benzimidazol – 2 – ylcarbarmate) is a systematic broad spectrum fungicide having chemical formula $C_9H_9N_3O_2$ and a molecular weight of 191.9. it is also obtained as degradation products of thiophoate- methyl and benomyl fungicides (Mazellier *et al*., 2003; Fang *et al*., 2012). Carbendazim is used worldwide as pre and post harvest treatment to control fungal diseases such as ascomycetes, fungal imperti and basidiomycetes on various fruits and vegetables (Tortella *et al*., 2013, Devi *et al*., 2015). In addition Carbandazin is used in paint, textile, paper and leather industries (Selmanoglu *et al*., 2001).

Carbandazim is classified in the hazardous category of chemical by World Health Organization. It is has been classified along with carbomyl as possible human carcinogens (Goodson *et al*., 2013). Carbandazim has been banned in several countries because of its negative impacts on the environment and health such as the development and reproductive disturbances, toxicity and mutagenicity (Costa *et al*., 2018). The adverse effects of carbendazim on the biochemical, histopathological and hematological parameters in the

kidney and their hormone levels have been demonstrated using rats as test subjects (Parag *et al*., 2011).

1.2 Aims and Objectives

Aim: The aim of this study is to investigate the differential effect of one and two weeks exposure to Carbendazim on renal toxicity in male rat.

Objectives

i. Estimation and assay of selected plasma metabolites such as urea and creatinine to check the effect of Carbendazim on the kidney of rats.

ii. Antioxidant parameters such as Superoxide Dismutase,Malondialdehyde and Catalase activity are estimated and assayed to check the enzyme activity and free radicals production in order to detect the effect of carbendazim on kidney of male rat.

1.3 Literature Review

1.2.1 Carbendazim

Carbendazim is a major metabolite of the fungicide benomyl and is also used as a fungicide itself. It is a systematic fungicide with activity against a number of plant pathogens. The fungicidal properties of carbendazim are the results of the binding of carbendazim to tubulins,an effect which disrupts microtubule formation and mitosis (Dustin, 1984).

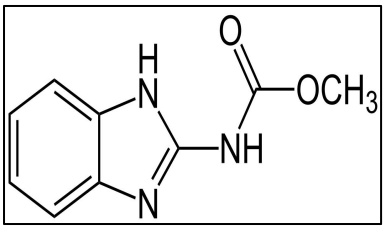
Carbendazim is a commonly used industrial fungicide with broad spectrum antifungal properties. It is commonly used in the control of fungal pathogen in cereals, vegetables, ornamental and fruit crops (Mahob *et al*., 2014). There are some evidence that the route of exposure of man and animals to carbendazim is via consumption of grains and fruits treated with the fungicide.

1.2.2 Physical properties of Carbendazim

Carbendazim is a white crystalline solid which melts at approximately – 250 ^{o}C and has a vapour pressure of <1x0^{-7} pa (< 1x 10^{-7}) mbar at 20^{o}C. Carbandazim is essentially insoluble in water (8 mg/liter solubility) at pH 7 and 20 ^{o}C. it is stable under normal storage condition (Shui-yan *et al*., 2004). It possess a density of 0.27 g/cm^{3} (loose) 10.62g/cm^{3} (packed)

1.2.3. Structure and chemical properties of Carbendazim

Carbendazim is the common name for methyl benzimidazol 2 – ylcarbamate or 2 methoxycarbonylamino benzimidazole (IUPAC)



Carbendazim has an empirical formular of $C_9N_9N_3O_3$, a relative molecular mass of 191.2. it slowly decomposes in alkaline solution (O'Neil, 2013). When heated to decomposition, it emits toxic fumes of nitric-oxide (Lewis, 2004). The Henry's law content = 1.5x10^{-12} atm – cum/mole at 25^{o}C.

1.2.4 Uses

Carbendazim is widely used to control fungal pathogens on various vegetables, fruits and several other plants (Tortella *et al*., 2013). Combination of carbendazim with mancozeb is used to effectively control sunflower leaf blight, chilli rots and mango anthracnose (Devi *et al*., 2015).

In addition, carbendazim is used in paint, textile, paper and leather industries as coating and preservation agents (Selmanoglu *et al*., 2001).

1.2.5 Route of exposure

General population exposure

The general exposure of carbendazim is through dietary intake or consumption of food treated with the fungicide (FAO/WHO, 1985).

Occupational exposure

Workers engaged in pesticide mixing and loading are exposed to dermal contact of few milligrams of benomyl per hour. This type of exposure is reduced by use of protective devices (EverHart and Holt, 1982).

1.2.6 Metabolism

1.2.6.1 Absorption

Carbendazim is well absorbed after oral exposure. Absorbed carbendazim is metabolized into many compounds within the organism. The main metabolites are 5HBC (methyl 5 – hydroxyl 1H benzimidazol 2 – yl) and 5, 6, HOBC (methyl 6 – hydroxyl N- oxo 5H benzimidazol 2 – yl) carbamate.

Minor metabolites are 5,6 DHBC-5 (6-hydroxyl 2(methoxY carbonyl)amino) 1H benzimidazol 5 – yl- 5 (hydrogen sulphate) 5,6 DHBC – G (B-D glocopyranosiduronic acid) (Krechniak and Klowsowka, 1986)

1.2.6.2 Transport and Storage

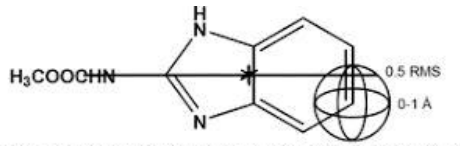
In small mammals, like rats and mice, After carbendazim oral administration, the highest concentration occur in the liver, it was distributed as carbendazim in the mitochondria, as SHB (Methyl 5 – Hydroxy 1H benzimdazol 2 –yl) in the cytosol and 2AB in the microsomes. Carbendazim and lite metabolites were also found in the kidney but no significant levels were detected in other tissues (Monson, 1990).

1.2.6.3 Biodegradation / transformation of Carbendazim

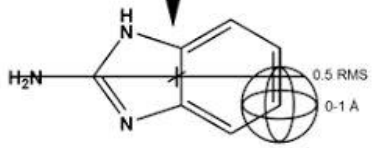
1.2.6.3.1 Photolytic degradation of carbendazim

Carbendazim applied on crops and fields reaches the soil ecosystem and subsequently disposed or transferred in different ways. It is usually transferred to the whole plant through roots, stem and leaves. As carbendazim is usually applied repeatedly at high doses to control fungal diseases and this is a serious environmental risk for humans, livestock, aquatic animal and soil microflora which needs immediate attention.

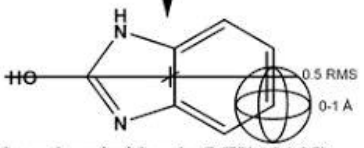
Photolytic degradation is one of the several pathways responsible for carbendazim derivatization. The hydroxyl radicals generated by UV photolysis of H_2O_2 are quenched by hydrogen carbonate and carbonate ions leading to formation of carbonate (CO_3^{2-} ions) which are highly efficient in carbendazim degradation (Manzelier *et al*., 2003). The degredation of carbendazim is also feasible via combination of TiO_2 – based photo-catalysis ozonation and UV light action (Rajeswari and Kanmani, 2009).



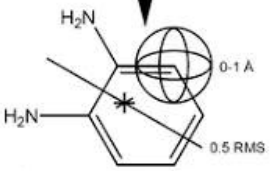
Methyl 1*H*-benzo[*d*]imidazol-2-ylcarbamate (MW 191.19)



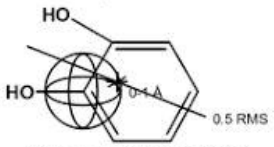
2-aminobenzimidazole (MW120.06)



2-hydroxybenzimidazole (MW 134.05)



1,2-diaminobenzene (MW 108.07)



Catechol (MW 110.04)

1.2.6.3.2. Microbial degradation of Carbendazim

Microbial degradation of carbendazim is affected by several biotic factors(presence of plants, competing microbes) and abiotic factors (pH, salt, soil types, humidity). Only few microbial strains are capable to tolerate and degrade carbendazim in situ or experimental conditions.

Bacteria and fungal usually cleave the methyl carbamate side chain of carbendazim parent structure leading to the generation of 2 – amino bezimidazole and 2 – hydroxyl benzimidezole derivatives.

Several strains of Rhodococus viz R. erythropolis CBII (Hotman and Kobayash, 1997). R. erythropolis CB-11 (Zhanz *et al*., 2013). R. jialingiae djI-6-2 (Wang *et al*., 2010 a, b) and *Rhodococcus* sp. (Jing-liang *et al*., 2006, Xiao *et al*., 2013) showed degradation of carbendazim in contaminated soils, laboratory pot experiment and in culture media. *Nocardiodes* sp., strain SG – 4G isolated from carbendazim contaminated area exhibited hydrolysis of carbendazim into 2 – amino – benzimidezole (Pandey *et al*., 2010).

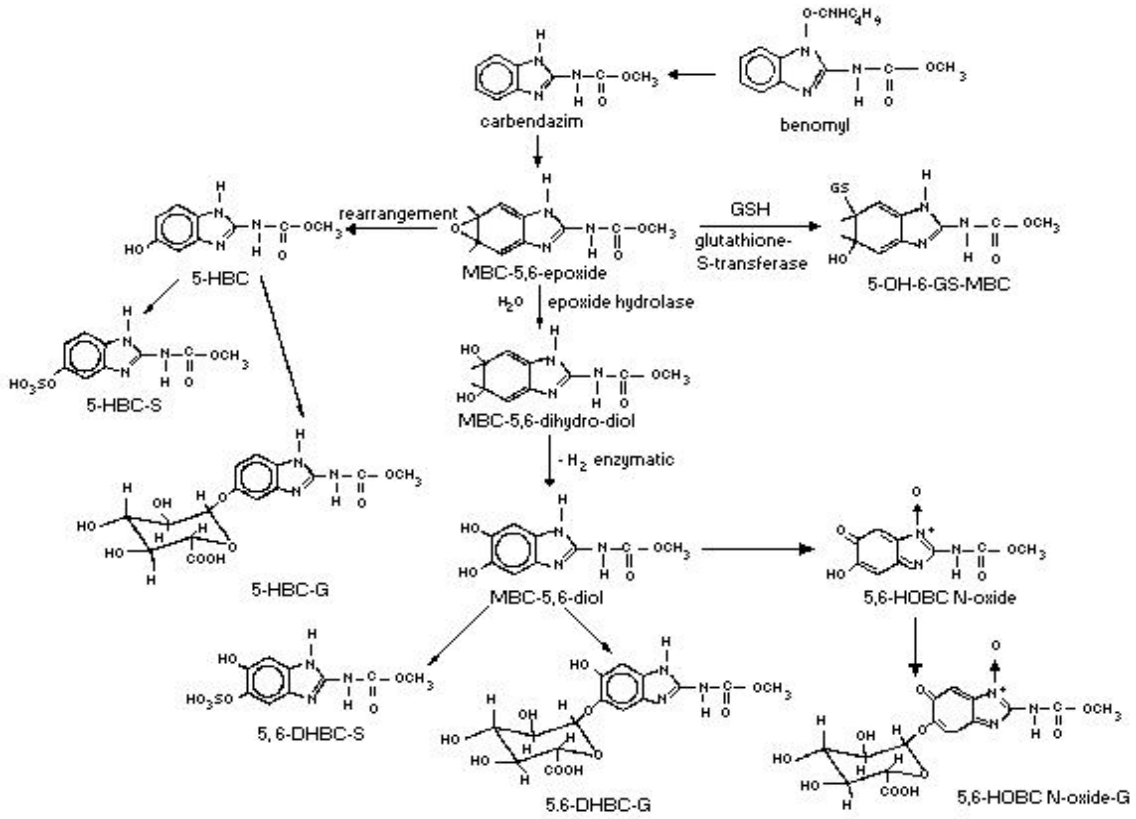


Fig. 1. Proposed metabolic pathway for carbendazim in rats (From: Monson, 1990)

1.2.6.3.3 Excretion

Carbendazim is excreted in urine and feaces within 72 hours after oral exposure / consumption (Monson, 1990).

1.2.7 Mechanisms of Toxicity

The mechanism of action of carbandazim and other benzimidazole compounds have shown that their biological effects result from their interaction with cell microtubules. These structures are involved in vital functions such as cell division and intracellular transport which are inhibited by carbendazim and benomyl.

The toxicity of carbendazim in mammals is linked to microtubule dyfunction .

1.2.8 Carbendazim and Kidney

The kidneys play a major role in the control of the consistency of the internal environment. The blood passing through the kidneys is first filtrated (glomerular filtration) so that all the constituents, except blood cells and plasma proteins, go into the microtubular systems. In the kidneys, the useful substances are quickly reabsorbed but unwanted substances escape filtration and are actively excreted in urine (El Sayed *et al*., 2013). However, renal function tests are important to identify renal dysfunction, to diagnose renal disease and to monitor disease progress. In medicine (nephrology) renal function is an indication of the state of the kidney and its physiological role in the body. Most doctors use the concentration of creatinine and urea to determine renal function. These measures are adequate to determine whether a patient is suffering from kidney disease (Munzir *et al*., 2015).

Sharma and Jain (2018) carried out histopathological examination of the kidney of carbendazim exposed rats, it showed congestion, necrosis and lesion, degeneration of convoluted tubules, reduction in the Bowman's space in kidney. Increased oxidative stress leads to diminished effectiveness of the antioxidant defense system (Flora *et al* 2012),Histopathological alterations in the kidney were mainly due to Carbendazim induced free radical injury.

1.2.9 Health impact of carbendazim

Carbendazim degradation results in the formation of 2 amino benzimdazole, a highly toxic component which binds to the spindle microtubules causing e nuclear division blockade (Yenjerla *et al*., 2009)

Carbendazim is known to manifest embryo toxicity, germ cell apoptosis, teretogenesis, infertility and developmental toxicity in different mammalian species (Yenjerla *et al*., 2009, Rama *et al*., 2014). Microtubules which are formed by non-covalent binding or α and β

tubulin are responsible for chromosome segregation during meiosis and mitosis (Rama *et al*., 2014). Tubulin exist invivo as α/β heterodimer and carbendazim is known to disrupt the dynamics of assembly and disassembly of microtubule.

Carbendazim inhibit the microtubule assembly which leads to impaired segregation of chromosomes during cell division (Pacheco *et al*., 2012, Rama *et al*., 2014). Experimental studies in mice and rats revealed that carbendazim causes hepatocellular dysfunction (Dikil *et al*., 2012, Saliho *et al*., 2015) affects hematopoiesis (Zubrod *et al*., 2014) increased the androgen receptor mRNA levels (Hsu *et al*., 2011), exerts endocrine disrupting effects (Lu *et al*., 2004; Banyova *et al* 2016) exerts oxidative stress in leydig cells (Correa *et al*., 2002; Rajeswari and Kanmi, 2009, Adedara *et al*., 2013; Sakr and Shalaby, 2014) and decrease the total white blood cell and platelets count (Shalibu *et al*., 2015).

Carbendazim disrupts the biochemical renal and hematological functions in mammals (Prashantkumar *et al*., 2012; Daundkar and Rampal, 2014). Carbandazim is reported to inhibit the prolification of murine and human cancer cell lines (Hammond *et al*., 2001; Yenjarla *et al*., 2009).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Materials

2.1.1 List of Chemicals

H_2O_2 (Gujarat Alkalies and Chemical, India), H_2SO_4 (Nutan Chemical, India) ,formalin (Huntsman International LLC, US) MDA reagent , Urea kit (Randox laboratories LTD), Creatinine kit (Fortress Diagnostics), SOD assay kit, Carbendazim (Sigma Aldrich).

2.1.2 List of Equipment

Water bath (Techmel and Techmel USA), Centrifuge (Model 80-2 Harris England), glasswares (Agary pharmaceuticals,Nigeria) gavage, weighing balance (metler analytical balance,Ohio,USA), micropipette (microlux, Spectrophotometer (Spectrumlab 22PC).

2.2 Method

2.2.1 Experimental Design

Twenty male rats (wistar strain) weighing between 191 +12.8 were obtained from the animal house of Animal and Environmental Biology, University of Benin. The animals were housed in well ventilated wire mesh cages. The animals were fed growers chow and water and acclimatized for a week.

2.2.2Animal Treatment

The rats were grouped into control and test group. The rats were labelled using picric acid and weight of individual rats were taken using a weighing balance and recorded as initial weight. The control group was orally administered 1% tween 80 using a gavage, while the

test group was orally administered 200mg/kg body weight of Carbendazim dissolved in 1% tween 80 (acts as vehicle) using a gavage. Oral administrations were done once and the rats were monitored for a period of 1-2 weeks and observations were recorded.

2.2.3 Animal Sacrifice

After one week of Carbendazim exposure, five (5) rats from each group were anaesthesized in chamber containing Chloroform and blood was collected by cardiac puncture. The kidneys were excised, trimmed free of connective tissues and weighed. One kidney from each group was immersed in formalin and used for histopatholigical study while the remaining kidneys were stored at -4^0c until needed for biochemical studies.

2.2.4 Preparation of Kidney Homogenate

The excised kidneys were homogenized in 5ml normal saline using mortar and pestle. The homogenate was centrifuged for 15min at 3500prm and the supernatant was collected and stored in the refrigerator.

2.2.5 Preparation of Plasma

The collected blood was centrifuged for 15min at 3500rpm and the plasma was collected and stored.

2.2.5 Preparation of Reagents

Urea reagent:

Sodium nitroprusside and urease: The contents of vial R1a was transferred into R1b bottle and mixed.

Phenol : The contents of bottle R2 was diluted with 660ml of distilled water and the bottle was thoroughly rinsed and mixed.

Sodium hypochloride (R3): the contents of bottle R3 was diluted with 750ml of distilled water.the bottle was rinsed and mixed thoroughly

30mm H_2O_2: Aliquot (0.34ml) of H_2O_2 was dissolved in 100 ml of 0.05m phosphate buffer of pH 7.0.

0.05m phosphate buffer (pH 7.0): Aliquot (4.36g) of disodium hydrogen phosphate and 2.72g of potassium dihydrogen phosphate were dissolved in 900 ml distilled water, the pH was adjusted to 7.0 using 0.1 m HCl and the resulting solution was made up to 1000 ml with distilled water.

0.1 m HCl: Aliquot (3.64ml) of 0.1 m HCl was added to 1000 ml distilled water.

Creatinine reagent: Equal quantities of sodium hydroxide and picric acid solution was mixed.

0.25N HCl: Aliquot (4.3ml) of conc. HCl was dissolved in distillled H_2O and the resulting solution was made up to 100 ml with distilled water.

6M H_2SO_4: Aliquot (163.2ml) of conc. H_2SO_4 (98% SP c – 1.84) was added to 600 ml of distilled water. The solution was gently stirred to prevent the formation of lower layer of acid and the volume made up to 1000 ml.

9% Saline solution (Normal saline): Aliquot (9g) sodium chloride was dissolved in 1000 ml distilled water.

0.01 m KMNO4: Aliquot (0.49g) of $KMnO_4$ was added to 500 ml distilled water.

TCA –TBA – HCl (MDA reagent): Aliquot (15.31g) of TCA and 0.375g of TBA were dissolved in 70 ml of 0.25N HCl, the volume was made up to 100 ml using the same 0.25 N HCl.

SOD Chromogen solution: The SOD chromogen diluent was added to the SOD chromogen powder.

1% Tween80: Aliquot (1ml) of tween80 was added into a 100ml volumetric flask and made up to the mark.

2.2.6. Antioxidant Assay Screening

2.2.6.1 Catalase (E.C 1.11.1.6)

Principle

Catalase is present in nearly all animal cells,plants and bacteria and acts to prevent accumulation of noxious peroxide which is converted to oxygen and water.

$2H_2O_2$ _____ $2H_2O + O_2$

At high concentrations of low molecular weight alcohols or formaldehyde and low peroxide concentration,catalase also exhibits peroxidase activityand can utilise as substrates,organic hydroperoxides with small aliphatic substituents such as ethyl hydrogen peroxide and peracetic acid. physiologically, there may exist high concentrations of other receptors and low concentration of peroxide,it is conceivable that catalases serves almost exclusively as a peroxidase in animal tissues.

Procedure

The method of Cohen *et al*. (1970) was adopted. 0.5ml of the homogenate supernatant added into ICC – Cro test tubes while the blank contained 05ml distilled water. The reaction was initiated by adding sequentially at fixed interval, 5ml of cold 30 mm H_2O_2 and mixing thoroughly by inversion. After exactly 3 minutes, the reactions were stopped sequentially at

the same fixed interval by rapidly adding 1 ml of 6 m H_2SO_4 and were mixed quickly by inversion.

The test samples and the blank were taken one at a time and 7 ml of 0.01m $KMNO_4$ was added which was mixed twice by inversion and absorbance at 480 nm read within 30 – 60 seconds. The spectrophotometric standard was prepared by adding 7 ml of 0.01 m $KMNO_4$ to a mixture of 5.5ml of 0.05 m phosphate burer pH 7.0 and 1 ml of 6Mm sulphuric acid. The spectrophotometer was zeroed with distilled H_2O. the acidity of was estimated.

Calculation

The decomposition of hydrogen peroxide H2O2 by catalase follows first order kinetics given by the equation

$$K = \log\frac{s0}{s3}\ x\frac{2.3}{t}$$

Where;

K= first order rate constant.

So= substrate concentration at zero time.

S3= substrate concentration at 3 minutes.

t = time interval over which the reaction is measured.

St= spectrophotometric standard.

SB= Absorbance of the reaction system of the blank.

S3= St-SA

SA= Absorbance of the reaction sample

2.2.6.2 Superoxide Dismutase

Principle

Superoxide dismutase has the ability to inhibit the autoxidation of pyrogallol.The autoxidation of pyrogallol in the presence of EDTA in the pH 8.2 is 50%.The principle of this method is based on the competition between the pyrogallol autoxidation by O2- and the dismutation of this radical by Superoxide Dismutase (SOD).

PROCEDURE

50µl of the test sample was added into a dean cuvette. 1ml of SOD assay buffer was added to the cuvette. 1 ml of SOD chromogen solution was also added to the cuvette and mixed. Distilled water (50 µl) was added to the blank. Absorbance was read immediately at 420 nm and then again after 1 minutes.

Calculation

$$inhibition\ of\ pyrogallol\ autoxidation = \frac{\Delta s}{\Delta c}\ x\ 100\%$$

$$SOD\ activity\ in\ v/ml\ = \frac{\%\ inhibition\ of\ pyrogallol\ autoxidation}{50\%}$$

2.2.6.3 Malondialdehyde Assay

Principle

Malondialdehyde is formed from the breadown of poly unsaturated fatty acid and membrane lipids to attack by free radicals which cause damage and compromise the integrity of the membrane . Malondialadehyde (MDA) is a convenient index for determining lipid

peroxidation. when heated with TBA under acid conditions , it forms a pink coloured product which has a maximum absorbance at 535nm.

Procedure

MDA level was estimated by the method of Burge and Aust 91978). An aliquot of the kidney homogenate (1 ml) was added to 2 ml of (1:1:1 v/v/v). Triichloroacetic acid (TCA), Thiobarbituric acid (TBA) and Hydrochloric acid (TBA 0.375% w/v), TCA acid 15% w/v) HCl 0.25m) and mixed thoroughly by swirling. The solution was heated for 15 minutes in a boiling water bath and then cooled. The floculed precipitate was removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the clear supernatant was measured against a standard or reference blank at 535 nm.

Calculation

$$\frac{absorbance\ of\ sample}{absorbance\ of\ standard}\ x\ concentration\ of\ standard$$

Standard concentration = 0.184 mg/dl

The resulting concentration was converted to mg/g tissue using the weight of the tissue.

2.2.7 Assay of Selected Plasma Metabolites

2.2.7.1 Urea

Principle

Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

Urea + H_2O ---------- $2NH_3 + CO_2$

NH_3 + hydrochloric +phenol ---------- indophenol (blue compound)

Procedure

Using the blood plasma, 10µl of sample (plasma) was pipetted into test tubes 10µl of distilled H2O was added to the reference blank. 10µl was added to each test tube. It was mixed and incubated at 37 ºC for 10 minutes.

After 10 minutes 2.5 ml of reagent 2 and 3 were added across each test tube. The mixture was mixed and incubated at 37ºC for 15 minutes.

Absorbance of sample and standard was read against the blank. The colour of the reaction is stable for at least 8 hours.

Calculation

$$Urea\ concentration\ = \frac{A\ sample}{A\ standard}\ x\ standard\ concentration$$

Urea standard = 79.27 mg/dl

Normal udss and serum values=10 – 55 mgl/dl

2.2.7.2 Creatinine

Principle

Creatinine reacts with picric acid in an alkaline medium to form a dep yellow complex. The amount of complex formed is directly proportional to the level of creatinine in the sample.

Procedure

Equal amount of NaOH and picric acid was mixed for the number of tests to be performed.

100µl of sample (plasma) was pipetted into test tubes 100 µl of standard was also pipetted into a test tube. 1 mil of working reagent was added across each test tubes.

It was mixed and the absorbance of sample and standard was read after 30 sec. then again after exactly 120 secs.

Calculations

$$Creatine\ conc.\ = \frac{\Delta\ abs\ sample}{\Delta\ Abs\ standards}\ x\ conc.\ of\ creatine\ standard$$

Creatinine standard concentration = 2mg/dl

Normal values

Men = 0.6 – 1.1 mg/dl

Women 0.50 – 0.90 mg/dl

2.2.8. Preparation of slides for Histopathology

Tissue processing procedure

The tissue was dehydrated in ascending grades of alcohol (70%,90% ,96%,and absolute with Atp). It was then dealcoholized (cleared) in xylene and impregnated in molten paraffin wax. To give it support, it was embedded in paraffin wax and sectioned onto a slide with the microtome. It was allowed to dry on the hot plate and prepared for staining.

Hematoxylin and Eosin staining procedure

The section was dewaxed in xylene and two changes each lasting 5min were observed. It was hydrated in descending grades of alcohol (absolute.96%,90%,70%) 5 Mon each, sections were watered and stained in Hematoxylin for 10min. After 10min,it was rinsed in water and differentiated briefly in 1% acid alcohol,for 10min,it was blue in warm water after which it was counter stained with Eosin for 1-3min and rinsed in water. It was then dehydrated in ascending grades of alcohol for 5min each. After which it was cleared or dealcoholized in Xylene and three changes were observed. Finally ,it was mounted in DPX (Dibutylphthalate polystyrene Xylene).

CHAPTER THREE

RESULT

3.1 Effect of Carbendazim exposure on rat kidney after 7 days and 14 days Respectively.

Data obtained for body weight of rats are shown on Table 1.

Results showed that after 7 days, there was a significant increase ($p<0.05$) in Body weight of rats treated with Carbendazim when compared to the Control .There was no significant ($p>0.05$) decrease in Body weight of animals treated with Carbendazim relative to the Control after 14 days.

Results also showed that the kidney/Body weight ratio was not significantly ($p>0.05$) decreased in Carbendazim treated rats relative to the Control after 7 days and 14 days.

There was no significant ($P>0.05$) increase in the concentrations of Catalase and Malondialdehyde in the test group relative to the control.

Superoxide Dismutase activity increased significantly ($p<0.05$) in Carbendazim treated rats relative to the control after 7 days ,after 14 days ,there was no significant increase ($p>0.05$) in SOD activity.(Data shown in Table 2)

Plasma metabolite Creatinine increased significantly ($p<0.05$) in the test group after 7days and there was no significant increase ($p>0.05$) after 14 days.There was a significant increase ($p<0.05$) in the concentration of Urea in the test group relative to the control after 7days and 14 days.(Data shown in Table 3)

Table 1: Effect of Carbendazim on Body weight of Rats.

Groups	weight gain(g)		Relative kidney body weight ratio (%) $X10^{-3}$	
	After 7 days	After 14 days	After 7 days	After 14 days
Control (1% Tween80)	-9.85± 2..85[a]	17.13±1.69[b]	7.31±0.26[a]	7.48±0.28[b]
Carbendazim (200mg/kg Body weight)	5.80±0.35[b]	11.90± 1.40[b]	6.73±0.68[a]	6.43±0.06[b]

Values are Mean±S.D (n=5)

Values on the same column with different alphabets differ significantly($p<0.05$)

Effect of Carbendazim on Antioxidant Parameters in the kidney(MDA, SOD AND CATALASE)

TABLE 2

Groups	MDA Level (mg/g weight tissue) ($x10^{-3}$)		SOD Activity (units/g weight tissue) ($x10^{2}$)		CAT Activity (units/g weight tissue)	
	After 7 days	**After 14 days**	**After 7 days**	**After 14 days**	**After 7 days**	**After 14 days**
Control (1% Tween 80)	1.13±0.21[a]	2.39±1.22[b]	1.52±0.08[a]	3.51±0.44[a]	6.62±0.34[a]	[a]6.47±0.29
Carbendazim (200mg/kg Body weight)	1.63±0.59[a]	2.51±0.63[b]	2.26±0.19[b]	4.85±0.96[b]	6.77±0.41[a]	[b]7.76±0.21

Values are Mean±S.D (n=5)

Values on the same column with different alphabets differ significantly($p<0.05$)

Effects of Carbendazim on Plasma Metabolites (urea and creatinine) in kidney.

Table 3

Groups	Concentration of Urea(mg/dl) X10		Creatinine Concentration (mg/dl)	
	After 7 days	**After 14 days**	**After 7 days**	**After 14 days**
Control (1% Tween 80)	3.35±0.59[a]	17.42±0.28[a]	0.67±0.17[a]	3.59±0.09[a]
Carbendazim (200mg/kg Body weight)	11.97±1.59[b]	16.49±0.17[b]	1.29±3.16[b]	3.80±0.11[a]

Values are Mean±S.D (n=5)

Values on the same column with different alphabets differ significantly($p<0.05$)

3.2 Histopathological studies

At the Carbendazim dose used in this study (200mg/kg wt), photomicrographs revealed significant ultrastructural changes in all the Carbendazim exposed rats relative to the control.This included interstitial inflammatory infiltrates and tubular necrosis.These histopathological alterations are indicated in plates 1-4;

I

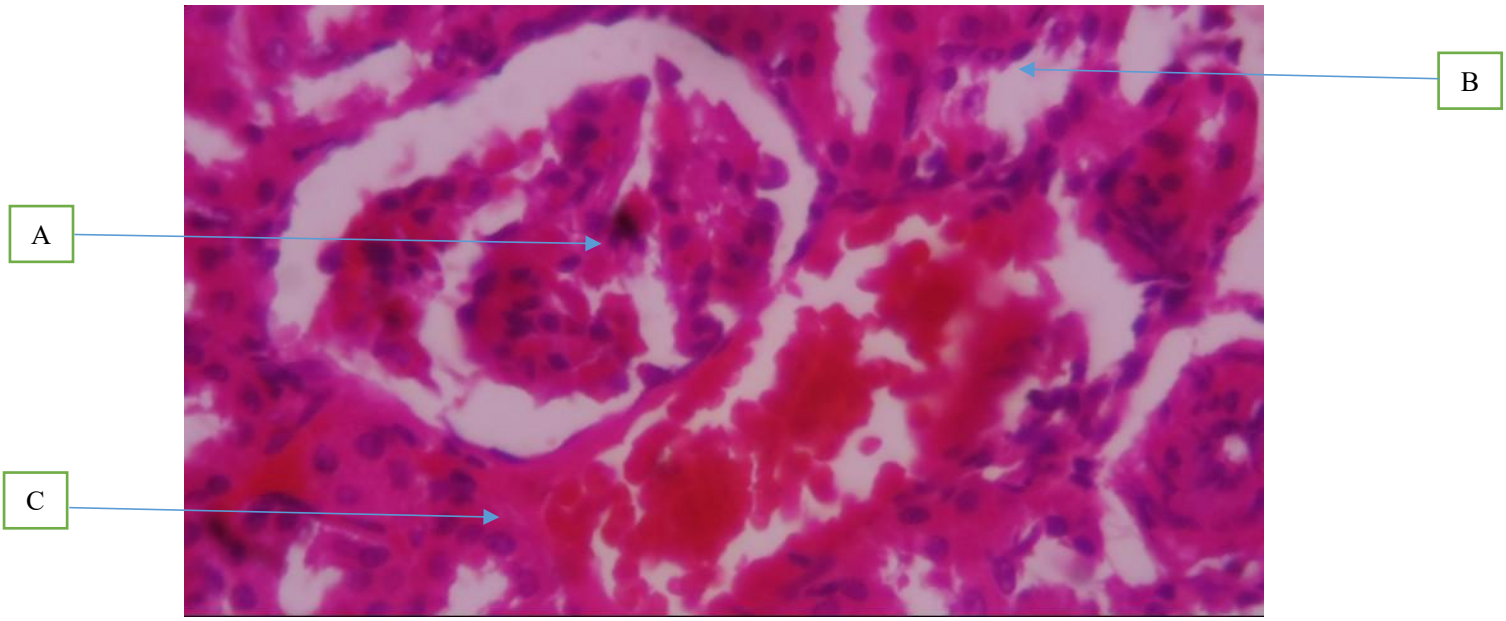


Plate 1: Histological section through the Kidney. Control. Wk 1. Composed of A: glomerulus, B, tubules and C, interstitial space (H&E x 400)

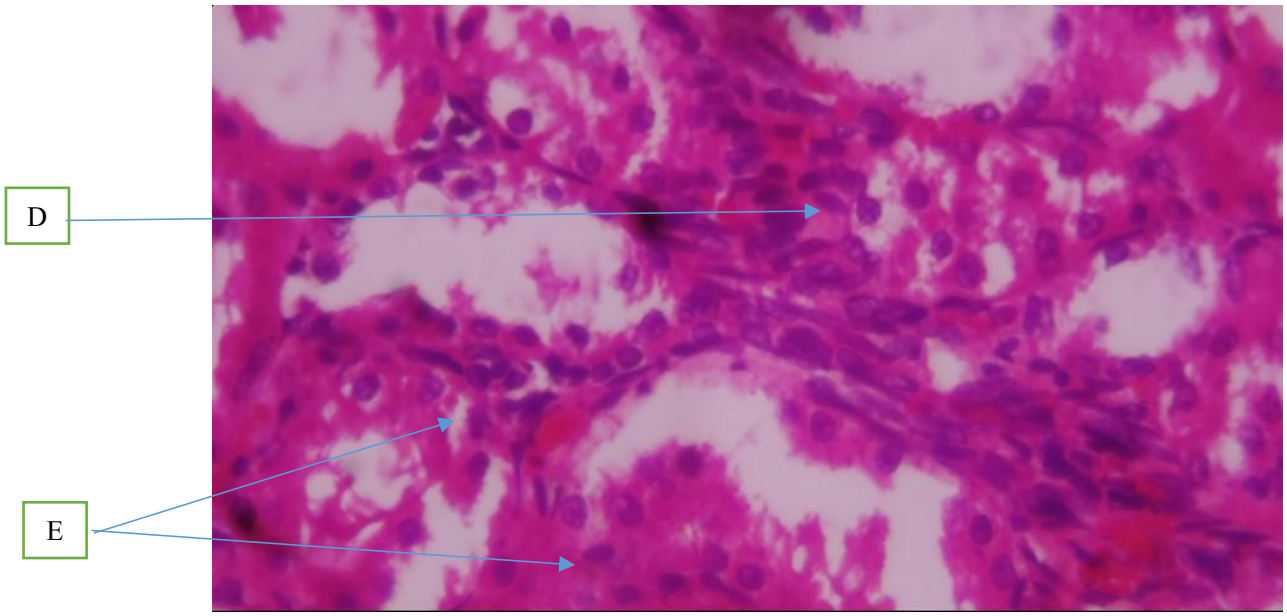


Plate 2: Histological section of kidney of Rat given Carbendazim. Wk 1: Showing D: interstitial inflammatory infiltrates and E: tubular necrosis (H&E x 400)

The glomerulus and tubules are well preserved in the kidney of control (plate 1).In constrast , histologic sections in (plate 2 and) show inflammatory infiltrates and tubular necrosis which indicates kidney impairment.

A; Tubules
B: Glomerulus
C; Interstitial space
D; Interstitial inflammatory infiltrates
E: Tubular necrosis

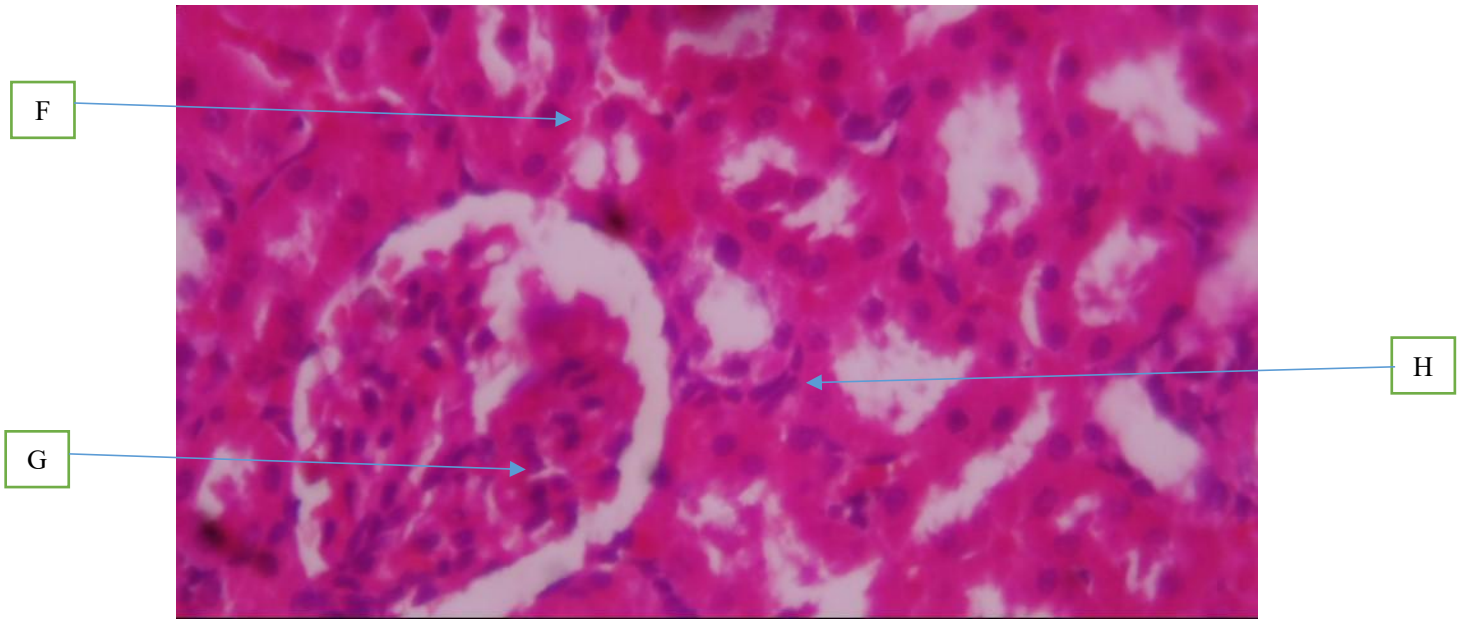


Plate 3: Histological section of Kidney. Control. Wk 2. Composed of: F:tubules, G; glomerulus and H: interstitial space (H&E x 400)

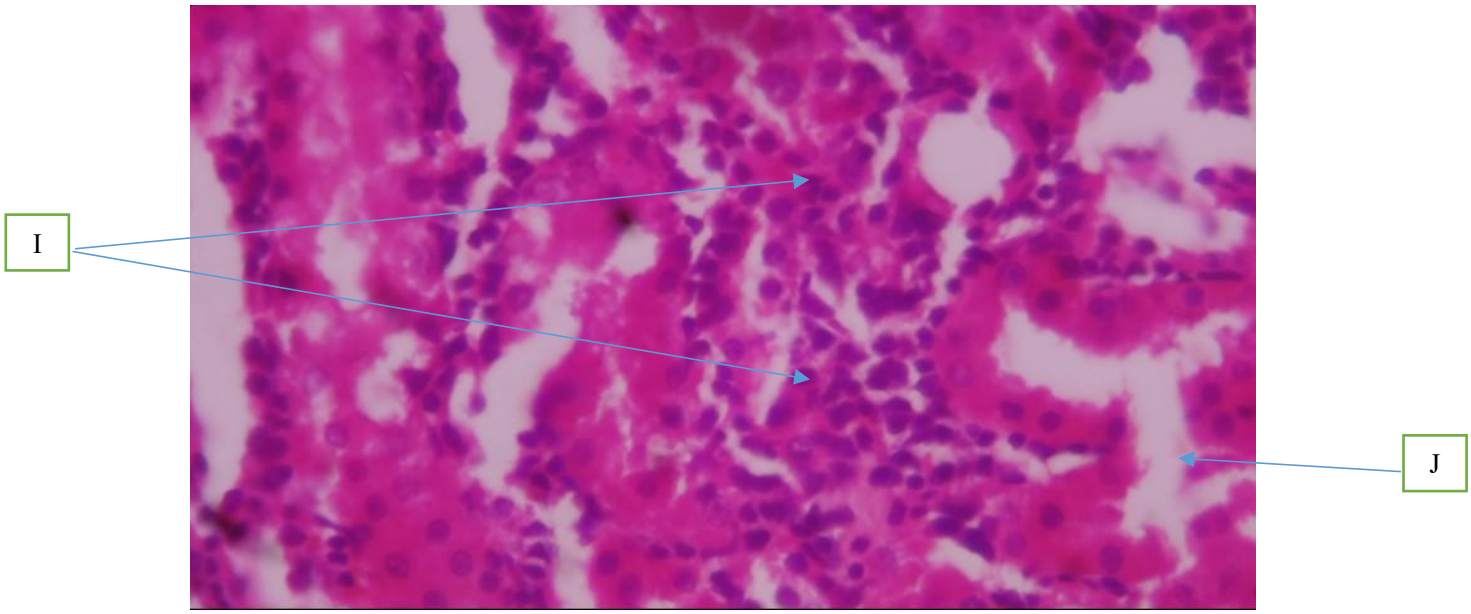


Plate 4: Histological section of kidney of Rat given Carbendazim. Wk 2: Showing I: interstitial inflammatory infiltrates and J: tubular necrosis (H&E x 400)

The glomerulus and tubules are well preserved in the kidney of control (plate 3).In constrast , histologic sections in (plate 4) show inflammatory infiltrates and tubular necrosis which indicates kidney impairment.

F; Tubules
G: Glomerulus
H; Interstitial space
I; Interstitial inflammatory infiltrates
J: Tubular necrosis

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1. Discussion

Humans or mammals may be exposed to Carbendazim via consumption of food products containing residues of benomyl or carbendazim. Administration of Carbendazim to rats elicited changes in biochemical parameters and the kidney histo-architecture of rats used in this study. There was a significant change ($p<0.05$) in the absolute body weight of rats treated with Carbendazim compared with control after 7 days but there was no significant change ($p>0.05$) in body weight after 14 days. We did not observe any significant decrease ($p>0.05$) in the relative kidney/body weight ratio of rats treated with Carbendazim relative to the control after 7days and 14days.

After 7 days of Carbendazim exposure, there was no significant change ($p>0.05$) in kidney weight of rats when compared with the normal control but the weight of kidney decreased significantly ($p<0.05$) after 14 days of Carbendazim exposure. Absolute weight of kidney is a sensitive predictor of nephrotoxicity (Seely,2017).

Malondialdehyde (MDA) is an index of tissue membrane degeneration and lipid peroxidation via free radical production (Veena *et al*,2007). Increase in MDA levels were not significant ($p>0.05$)in carbendazim treated groups compared with normal control after 7days and 14 days respectively. Increase in MDA levels might indicate oxidative stress. This result is in constrast with the report of Nwozo *et al.* (2017). The difference in results might be due to diet. The elevation in Catalse activity after 7 days was not significant($P>0.05$) in Carbendazim treated group when compared to the control but Catalase activity increased significantly ($P<0.05$)after 14 days which may be an indicator of the body trying to alleviate oxidative stress.

Superoxide Dismutase has the ability to inhibit the autoxidation of pyrogallol. It has been observed that increased Superoxide Dismutase (SOD) is beneficial in event of increased free radical production (Pham-Huy *et al* 2008). SOD levels increased significantly ($p<0.05$) in carbendazim treated rats compared with the control after 7days of Carbendazim exposure. After 14 days, there was no significant increase in SOD activity of rats treated with Carbendazim. These observations in CAT and SOD in the kidney indicate an imbalance in the antioxidant system in the animals ,thus leading to increased peroxidation of membrane in the organs. The antioxidant system plays a critical role in protecting tissues (Oschsendorf, 1999).

Urea and Creatinine are renal function markers. The significant increase in Urea and Creatinine levels in the blood may be attributed to Carbendazim toxicity (Abolaji *et al*., 2017) and poor clearance of creatinine by the kidney, thus decreasing the renal elimination of these metabolic wastes .Urea levels increased significantly ($p<0.05$) in carbendazim treated group compared with the control after 7 days of carbendazim exposure. After 14 days,Urea levels decreased significantly ($p<0.05$) in carbendazim treated group. Creatinine levels increased significantly ($p<0.05$) after 7 days in carbendazim treated group compared with the normal control. After 14 days, the increase in Creatinine levels was not significant ($p>0.05$) in Carbendazim treated group compared with control. This result is consistent with the report of Nwozo *et al* (2017). The kidney maintains the blood creatinine levels in a normal range. Elevated creatinine level signifies impaired kidney function. Urea is the chief nitrogenous end product of the metabolic breadown of proteins in all mammals and it is passed to the kidney and excreted in the urine, so increased levels signifies renal impairment.

In histopathological examination, Ultra-structural changes were observed in the kidney of rats treated with carbendazim compared to those of control. Inflammatory infiltrates and tubular necrosis which indicates damage to kidney tubules were observed in the kidney of

carbendazim treated rats (plate 2 and plate 4) after 7 days and 14days respectively. This observation is in accordance with the histopathological investigation carried out by Selmanoglu (2001).

According to the results of this study, Carbendazim caused differences in biochemical and Histopathological damages in the kidney of rats.

4.2 Conclusion

The findings from this study suggests that upon intoxication with Carbendazim at a dose of 200 mg/body weight, Carbendazim caused changes in body weight of rats.It also induced changes in biochemical markers of oxidative stress such as Catalase, Superoxide Dismutase and Malondialdehyde.Urea and Creatinine which are significant markers of renal function were increased significantly which indicates renal impairment caused by Carbendazim exposure.As the demand for pesticides is rapidly increasing worldwide, environmental accumulation, contamination and life threatening effects on living organisms is inevitable.The increasing use of carbendazim and it's serious toxic effects are considered an ever-increasing threat to the ecological balance of the environment. It is suggested that the use of pesticides in countries where pesticides are widely used should be properly regulated and controlled.

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APPENDIX

Calculation of dosage of 1% tween80 administered to rats.

To prepare 1% Tween80, using mean weight of biggest rat from the groups,

$\frac{227.2+237.2+223.0}{3}$=**229.14g**

Taking average unit of the biggest rat weight from the groups

$$\frac{5.06 + 26.09 + 19.35}{3} = 16.83\ units$$

229---------- 16.83 units

Z ---------X

Z=$\frac{Z\ x\ 16.83}{229.14}$**=0.073X**

labels	Weight (g)	Amount dispensed Z=0.073X
1	165.50	12.08
2	223.20	16.29
3	179.3	13.09
4	205.9	15.03
5	174.2	12.72
6	217.0	15.84
7	198.70	14.51
8	170.8	12.47
9	195.60	14.28
10.	176.80	12.91

CALCULATION OF DOSAGE OF CARBENDAZIM ADMINISTERED TO RATS

Treatment dose ----200mg/kg body weight

1000g---------200mg

X ---------- y

Y=$\frac{200\ x\ X}{1000} = 0.2x$

IF 72mg------ 40 units

Y -------- Z

Z=$\frac{40\ x\ Y}{72} = 0.55\ Y$

LABELS	WEIGHT (g)	AMOUNT OF TOXICANT Y =0.2X	AMOUNT DISPENSED Z=0.55Y
1	237.20	47.44	26.36
2	204.40	40.88	22.48
3	195.20	39.04	21.47
4	205.3	41.06	22.58
5	206.10	41.22	22.67
6	161.9	32.38	17.81
7	179.4	35.88	19.73
8	180.10	36.02	19.81
9	164.00	32.80	18.04
10	181.40	36.28	19.95

CALCULATION OF MEAN, STANDARD DEVIATION AND T-TEST OF UREA FOR 7 DAYS AND 14 DAYS RESPECTIVELY

UREA	**WEEK 1**		**WEEK2**	
	CONTROL(UREA)X10 mg/dl	**CARBENDAZIM (UREA)X10 mg/dl**	**CONTOL (UREA)X10 mg/dl**	**CARBENDAZIM (UREA)X10 mg/dl**
1	3.43	13.54	17.46	16.46
2	3.89	12.03	17.12	16.68
3	2.71	10.34	17.68	16.35
$\sum X$	10.01	35.90	52.26	49.49
$\overline{X}$	3.35	11.97	17.49	16.49
$\sum (X - \overline{X})2$	7.09	51.19	1.54	0.57
S.D	0.59	1.59	0.28	0.17

Week 1

T-TEST

MEAN DIFFERENCE= $\overline{X1}$- $\overline{X2}$

33.45 -119.68 =-86.22

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= $\sqrt{\frac{511.36+70.24}{4}}$ =12.07 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 12.07 x 0.8165=9.86

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =$\frac{86.22}{9.86}$=8.74

Ttable (0.05 ,DF=4) =2.776

WEEEK 2

MEAN DIFFERENCE= $\overline{X1}$- $\overline{X2}$

174.19 -164.99 =-9.2

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= $\sqrt{\frac{15.46+5.64}{4}}$ 2.29 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 2.29 x 0.8165=1.88

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =$\frac{9.2}{1.88}$=4.91

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is greater than Ttable and as such there is a significant difference.

Calculation of mean and standard deviation of creatinine

Creatinine	WEEK 1		WEEK2	
	CONTROL(creatinine) Mg/dl **X**	**CARBENDAZIM(creatinine) md/dl** **X**	**CONTROL (creatinine) mg/dl** **X**	**CARBENDAZIM (creatinine)mg/dl** **x**
1	0.857	**1.429**	3.58	3.70
2	0.571	**1.429**	3.68	3.92
3	0.571	**1.143**	3.50	3.78
4		**1.143**		
$\sum X$	**1.999**	5.144	10.76	11.40
$\bar{X}$	0.670	1.286	3.59	3.80
$\sum (X - \bar{X})2$ X10^{-2}	5.50	8.20	1.63	2.5
S.D	0.765	0.165	0.09	0.11

Week 1

T-TEST

MEAN DIFFERENCE= $\overline{X1}$- $\overline{X2}$

0.67-1.286 =-0.616

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}} = \sqrt{\frac{2(0.16)2+3(0.17)2}{5}}$=0.166 n1=3 ,n2=4

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.166x 0.764=0.127

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==0.616/0.127=4.85

T-table (0.05 ,DF=5) =2.571

Conclusion: T calculated is greater than Ttable and as such we reject the hypothesis,i.e there is a significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

=3.8 -3.59=0.21

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ = $\sqrt{\frac{2(0.11)2+3(0.09)2}{4}}$= 0.10 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 0.10 x 0.8165=0.082

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$=0.21/0.082=2.56

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

SOD	WEEK 1		WEEK2	
	CONTROL(SOD)x 10^2	**CARBENDAZIM(SOD) X10^2**	**CONTOL(SOD)X 10^2**	**CARBENDAZIM (SOD)X10^2**
1	1.46	2.18	3.05	5.40
2	1.61	2.48	3.57	3.75
3	1.48	2.12	3.92	5.41
$\sum X$	**4.55**	6.78	10.54	14.56
$\bar{X}$	1.52	2.26	3.51	4.85
$\sum(X-\bar{X})2$ X10^{-2}	1.3	7.4	0.38	1.83
S.D	0.08	0.19	0.44	0.96

Week 1

T-TEST

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

1.52-2.26 =-0.74

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ = $\sqrt{\frac{2(0.19)2+2(0.08)2}{4}}$ = 0.096 n1=3 ,n2=3

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.096 x 0.8165=0.079

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$=0.74/0.0785=9.43

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is greater than Ttable and as such we reject the hypothesis,i.e there is a significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

3.51 -4.85=-1.34

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= $\sqrt{\frac{2(0.96)2+2(0.44)2}{4}}$0.747 n1=3 ,n2=3

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.747 0.8165=0.609

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$=1.34/0.609,=2.036

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

CALCULATION OF MEAN, STANDARD DEVIATION AND T-TEST OF MDA

MDA	**WEEK 1**		**WEEK2**	
	CONTROL(MDA)x10 -3	**CARBENDAZIM(MDA)X10-3**	**CONTOL(MDA) X10-3**	**CARBENDAZIM (MDA)X-3**
1	1.26	1.95	1.99	2.41
2	1.05	1.98	1.42	2.00
3	1.07	0.95	3.76	3.13
$\sum X$	3.83	4.88	7.17	7.54
$\bar{X}$	1.13	1.63	2.39	2.51

$\sum(\boldsymbol{X}-\overline{\boldsymbol{X}})\mathbf{2}$	2.69x10-8	6.80x10-4	2.98x10-6	8.05x10-7
S.D	0.12	0.59	1.22	0.63

Week 1

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

1.13-1.63 =-0.50

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= = 0.443 n1=3 ,n2=3

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.443 x 0.8165=0.362

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ ==0.50/0.362=1.38

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

2.39 -2.51=-0.12

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$=0.974 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 0.974 0.8165=0.795

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ ==0.12/0.795=0.151

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF CATALASE

CAT	**WEEK 2**		**WEEK 1**	
	CONTROL(CAT)	**CARBENDAZIM(CAT)**	**CONTOL(CAT)**	**CARBENDAZIM (CAT)**
1	6.22	7.99	6.28	6.85
2	6.78	7.69	6.63	6.34
3	6.41	7.59	6.95	7.14
$\sum X$	19.41	23.27	19.87	20.32
$\bar{X}$	6.47	7.76	6.62	6.77
$\sum (X - \bar{X})2$	0.17	0.086	0.22	0.34
S.D	0.29	0.21	0.34	0.41

Week 1

T-TEST

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

6.62-6.77=-0.15

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ =0.38 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 0.38 x 0.8165=0.308

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==0.15/0.308=0.487

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such there is no significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

6.47 -7.76=-1.29

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 0.253 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 0.253 x 0.8165=0.207

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =1.29/0.207=6.23

Ttable (0.05 ,DF=4) =2.776

Conclusion: T calculated is greater than Ttable and as such there is a significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF WEIGHT GAIN

WEIGH GAIN	**WEEK 1**		**WEEK2**	
	CONTROL(weight gain)	**CARBENDAZIM(weight gain)**	**CONTOL(weight gain)**	**CARBENDAZIM (weight gain)**
1	-7.00	5.30	15.8	10.50
2	-12.70	6.30	17.7	**13.30**
3	-9.85	5.80	**17.9**	**11.9**
$\sum \boldsymbol{X}$	-29.55	17.4	**51.40**	35.7
$\bar{\boldsymbol{X}}$	-9.85	5.8	17.13	11.90
$\sum(\boldsymbol{X}-\bar{\boldsymbol{X}})\mathbf{2}$	16.25	0.25	5.71	3.92
S.D	2.85	0.35	1.69	**1.40**

Week 1

T-TEST

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

-9.85 -5.80 =-15.65

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= =2.03 n1=3 ,n2=3

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 2.03x 0.8165=1.66

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$=15.65/1.66=9.43

Ttable (2) (0.05 ,DF=4) =2.776

Conclusion: T calculated is greater than Ttable and as such there is a significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

17.13-11.90 =5.23

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 1.55 n1=3 ,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 1.55x 0.8165=2.37

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==5.23/2.37=2.207

Ttable (2)(0.05 ,DF=4) =2.776

Conclusion: T calculated is less than Ttable and as such there is no significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF RELATIVE KIDNEY BODY WEIGHT RATIO

UREA	**WEEK 1**		**WEEK2**	
	CONTROL(Relative kidney body weight ratio)x10-3	**CARBENDAZIM(relative kidney body weight ratio)x10-3**	**CONTOL(relative kidney body weight ratio)x10-3**	**CARBENDAZIM (relative kidney body weight ratio)x10-3**
1	7.35	6.38	7.25	6.50
2	7.00	6.54	7.40	6.43
3	7.63/7.25	7.73/6.25	7.79	6.35
$\sum \boldsymbol{X}$	22.44	26.91	29.56	6.45
$\bar{\boldsymbol{X}}$	7.31	6.73	7.48	25.73
$\sum(\boldsymbol{X}-\bar{\boldsymbol{X}})\mathbf{2}$	0.20	1.38	0.16	0.01
S.D	0.26	0.68	0.28	0.062

T-TEST

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

7.31 -6.73=0.58x10-3

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= =0.51 n1=4 ,n2=4

Standard error= sp $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$X = 0.51x 0.7071=0.36

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =0.58x10-3/0.364x20-3=1.59

Ttable (2) (0.05 ,DF=6) =2.447

Conclusion: T calculated is less than Ttable and as such there is no significant difference.

WEEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

7.48-6.43=1.05

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 0.22 n1=4,n2=3

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 0.22x 0.764=0.168

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==1.05/0.168=6.25

Ttable (2)(0.05 ,DF=5) =2.571

Conclusion: T calculated is greater than Ttable and as there is a significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF INITIAL WEIGHT

UREA	**WEEK 1**		**WEEK2**	
	CONTROL(initial weight)x10	**CARBENDAZIM(initial weight)x10**	**CONTOL(initial weight)x10**	**CARBENDAZIM (initial weight)x10**
1	22.32	20.44	16.55	18.14
2	20.59	20.53	17.68	18.01
3	21.70	20.61	17.42	16.19
4	19.87	17.94	17.08	16.40
5	19.56	23.72	17.93	19.52
$\sum \boldsymbol{X}$	104.0	103.24	86.66	88.26
$\bar{\boldsymbol{X}}$	20.80	20.65	17.32	17.65
$\sum(\boldsymbol{X}-\bar{\boldsymbol{X}})\mathbf{2}$	55.67	168.29	11.61	75.61
S.D	1.18	2.05	0.54	1.38

T-TEST

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

20.88-20.65=2.32

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ =0.51 n1=5 ,n2=5

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 16.73x 0.632=10.58

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$=2.32/10.58=0.23

Ttable (2) (0.05 ,DF=8) =2.306

Conclusion: T calculated is less than Ttable and as such there is no significant difference.

WEEK 2

MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

17.33-17.65=-3.2

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ 10.44 n1=5,n2=5

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 10.44x 0.632=6.60

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ ==3.2/6.60=0.485

Ttable (2)(0.05 ,DF=8) =2.306

Conclusion: T calculated is less than Ttable and as such there is no significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF FINAL WEIGHT

FINAL WEIGHT	**WEEK 1**		**WEEK2**	
	CONTROL(final weight)x10	**CARBENDAZIM(final weight)x10**	**CONTOL(final weight)x10**	**CARBENDAZIM (final weight)x10**
1	21.62	20.97	18.72	18.80
2	20.32	21.16	19.26	19.06
3	22.16/	20.63	19.19	18.39
4	18.60	20.25	18.87	19.28
5	20.46	24.92	18.77	20.85
$\sum \boldsymbol{X}$	103.16	107.93	94.81	96.38
$\overline{\boldsymbol{X}}$	20.63	21.59	18.96	19.28
$\sum (\boldsymbol{X} - \overline{\boldsymbol{X}})\mathbf{2}$	15.67	143.75	2.45	35.36
S.D	1.38	1.89	0.25	0.94

T-TEST
MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

20.63 -21.59 =-9.54

SP ==$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$ =16.56 n1=5,n2=5

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 16.56x 0.632=10.47

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =9.54/10.47=0.91

Ttable (2) (0.05 ,DF=8) =2.306

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

WEEK 2
MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

18.96-19.28=-3.14

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 6.87 n1=5,n2=5

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$= 6.87x 0.632=4.35

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==3.14/4.35=0.72

Ttable (2)(0.05 ,DF=8) =2.306

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

CALCULATION OF MEAN ,STANDARD DEVIATION AND T-TEST OF KIDNEY WEIGHT

KIDNEY WEIGHT	**WEEK 1**		**WEEK2**	
	CONTROL(kidney weight)	**CARBENDAZIM(kidney weight)**	**CONTOL(kidney weight)**	**CARBENDAZIM (kidney weight)**
1	1.59	1.34	1.36	1.22
2	1.42	1.38	1.37	1.23
3	1.69	1.59	1.42	1.17
4	1.35	1.37	1.47	1.24
$\sum \boldsymbol{X}$	6.05	5.58	5.62	4.86
$\bar{\boldsymbol{X}}$	1.51	1.40	1.41	1.22
$\sum (\boldsymbol{X} - \bar{\boldsymbol{X}})\mathbf{2}$	0.07	0.06	7.99x10-3	3.31x10-3
S.D	0.16	0.14	0.052	0.03

T-TEST
MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

1.51-1.40=0.11

SP = $\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 0.15 n1=4 ,n2=4

Standard error= sp X $\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.15x 0.7071=0.105

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$ =0.11/0.105=1.11

Ttable (2) (0.05 ,DF=6) =2.447

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.

WEEK 2
MEAN DIFFERENCE=$\overline{X1}$- $\overline{X2}$

1.41-1.22=0.19

SP =$\sqrt{\frac{(n1-1)s1^2+(n2-1)s2^2}{n1+n2-2}}$= 0.04 n1=4,n2=4

Standard error= sp X$\sqrt{\frac{1}{n1}+\frac{1}{n2}}$ = 0.04x 0.7071=0.03

T= $\frac{MEAN\ DIFFERENCE}{STANDARD\ ERROR}$==0.19/0.042=4.42

Ttable (2)(0.05 ,DF=6) =2.447

Conclusion: T calculated is less than Ttable and as such we accept the hypothesis,i.e there is no significant difference.