# EFFECT OF *Entandrophragma utili* ON OXIDATIVE STRESS INDICES OF THE TESTES OF $CCl_4$ INTOXICATED WISTAR RATS

**BY**

**OJEABU DUKE OSAKPOLOR**

**LSC2103789**

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY**

**FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN**

**IN PARTIAL FULFILMENT OF THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc)**

**IN BIOCHEMISTRY**

**OCTOBER, 2025.**

## CERTIFICATION

This is to certify that this project report titled EFFECT OF *ENTANDROPHRAGMA UTILI* ON OXIDATIVE STRESS INDICES OF THE TESTES OF $CCL_4$ INTOXICATED RATS was presented by Ojeabu Duke Osakpolor with the matric number **LSC2103789** of the department of biochemistry, Faculty of Life Science, University of Benin, in partial fulfilment for the award of Bachelor of Science, (B.Sc.) in biochemistry.

**DR. K. ORUMWENSODIA** **DATE**

**(PROJECT SUPERVISOR)**

**DR. S. I. OJEABURU** **DATE**

**(PROJECT COORDINATOR)**

**PROF. E. C. ONYENEKE** **DATE**

**(HEAD OF DEPARTMENT)**

**EXTERNAL SUPERVISOR** **DATE**

## DEDICATION

This project is dedicated to God almighty, the giver of life and wisdom. I also dedicate it to my parents Mr. Assurance Ojeabu and Mrs. Patience Ojeabu for their love, encouragement and financial support. I also dedicate my work to myself for not giving up and believing in myself.

**ACKNOWLEDGEMENT**

I want to thank God the giver of life and wisdom and also acknowledge with deep sense of gratitude an inevitable, meticulous correction and intentionally guidance of Dr. Kissinger Orumwensodia my project supervisor in spite of his tight academic schedule made a huge support to my academic success.

I wish to express my profound gratitude and appreciation to all those who in one way or the other created the circumstances which led to an academic accomplishment, to my HOD Prof. E. C. Onyeneke, my project coordinator Dr. S. I. Ojeaburu, my course adviser, staff and non staff.

My heartfelt gratitude and appreciation goes to my parents Mr. Assurance Ojeabu and Mrs. Patience Ojeabu and to my siblings for their love and support. I also appreciate my colleague in the group for their support and understanding and also Mr. Richard Oseh Osehodion for his advantageous contribution to my work.

## TABLE OF CONTENT

## LIST OF FIGURES



## LIST OF PLATES

## ABSTRACT

Carbon tetrachloride ($CCl_4$) is a potent environmental toxicant that induces oxidative stress in the testes via reactive oxygen species (*ROS*) generation, leading to lipid peroxidation, antioxidant depletion, and impaired spermatogenesis. This study investigated the protective effect of *Entandrophragma utili* bark extract (*EUE*) on oxidative stress indices in the testes of $CCl_4$-intoxicated Wistar rats.

Thirty (25) male Wistar rats (150–180 g) were divided into five groups (n=5):

Group I: Normal control (distilled water)

Group II: $CCl_4$ only (0.5 mL/kg, 1:1 in olive oil, ip., twice weekly)

Group III: $CCl_4$ + Crude extract (400 mg/kgbwt)

Group IV: $CCl_4$ + Ethyl acetate (400 mg/kgbwt)

Group V: $CCl_4$ + Ethanol residue (400 mg/kgbwt)

Treatment lasted 28 days. Testes were excised, homogenized, and assayed for reduced glutathione (*GSH*), superoxide dismutase (*SOD*), catalase (*CAT*), and glutathione peroxidase (*GPx*). *Entandrophragma utili* bark extract exhibits dose-dependent antioxidant protection against $CCl_4$-induced testicular oxidative damage, possibly via free radical scavenging. It holds promise as a natural therapeutic agent for oxidative stress-related male reproductive disorder.

$CCl_4$ intoxication significantly ($p < 0.05$) decreased the activities of SOD, CAT, and GPx, and reduced GSH levels, confirming severe oxidative stress. Pre-treatment with the crude *EUE* extract significantly ($p < 0.05$) attenuated these alterations, restoring antioxidant enzyme activities and GSH levels near to normal and preserving testicular cytoarchitecture. The solvent fractions showed milder, non-significant restorative effects.

# CHAPTER ONE

## 1.0 INTRODUCTION AND LITERATURE REVIEW

### 1.1 INTRODUCTION

Male reproductive health remains a global challenge, increasingly affected by exposure to environmental toxins, poor nutrition, oxidative stress, smoking and other lifestyle related factors. About 7-15% of the global population has showed significant result of infertility, with male factors contributing to 50% of cases (Vander Borght and Wyns, 2023). It has been brought to light that oxidative stress has emerged as a major contributor, leading to testicular dysfunction, sperm damage and hormonal imbalance. Due to the high rate of cell division, rich content of polyunsaturated fatty acids in the sperm membrane, which are susceptible to peroxidation, and relatively low antioxidant capacity, the testes has become vulnerable to oxidative damage (Atiken, 2022).

Environmental toxicants like carbon tetrachloride ($CCl_4$), a known hepatoxin, that is toxic to reproductive organs induce testicular oxidative stress/damage by generating *trichloromethyl* radicals (-$CCl_3$) and *trichloromethyl peroxyl* (-$OOCCl_3$), via cytochrome P450 metabolism initiating lipid peroxidation and damage to testicular tissues in animal model.

In testicular tissues, $CCl_4$ exposure has been seen to reduce antioxidant enzyme activity (e.g., *GSH*, *SOD*, *CAT*) and increases malondialdehyde levels. The search for safer and more effective antioxidants has led to a renewed interest in medicinal plants (Ojo *et al.*, 2023).

Medicinal plants offer a promising approach to counter oxidative stress due to their rich phytochemical contents and as such they are increasingly studied for their antioxidant potential (Seven *et al.,* 2020).

*Entandrophragma utile* (Meliaceae family), is a tropical African plant widely used in traditional medicine for treating inflammation, ulcers, rheumatism and sickle cell disease. Its bark extract contains triterpernoids, limonoids, and flavonoids, exhibiting potent antioxidant activity (Olowosulu *et al.,* 2005; Falodun *et al.,* 2010).

### 1.1.1 JUSTIFICATION OF STUDY

The testes are highly susceptible to oxidative damage due to their high metabolic activity and abundance of polyunsaturated fatty acids (Atiken, 2022; Al-Daghistani *et al.,* 2023). Carbon tetrachloride ($CCl_4$) intoxication induces severe oxidative stress, impairing testicular function and fertility (Ojo *et al*., 2023). While synthetic antioxidants exist, they often have side effects, necessitating safer, natural alternatives.

*Etandrophragma utili* is a medicinal plant with reported antioxidant properties, but its protective effects on $CCl_4$-induced testicular oxidative stress remain underexplored. This study seeks to evaluate the impact of $CCl_4$ on testicular oxidative stress markers, determine whether *E.utili* extract can mitigate oxidative damage and preserve testicular function and provide scientific evidence for its potential use in managing oxidative stress-related infertility.

### 1.1.2 AIM AND OBJECTIVES OF THE STUDY

This present study investigates the effects of *Entandrophragma utili* extracts on $CCl_4$-induced testicular toxicity.

### 1.1.3 SPECIFIC OBJECTIVES OF STUDY

The specific objectives of this study includes, to determine;

- the effect of E*ntandrophragma utili* of testicular oxidative stress indices (*SOD*, *CAT*, *MDA* and *GSH*) in rats exposed to $CCl_4$.
- the restorative potential of E*ntandrophragma utili* on testicular architecture.

## 1.2.0 LITERATURE REVIEW

### Medicinal plants

Medicinal plants have long been integral to healthcare systems *Worldwide* and remain essential especially in developing nations. Reports indicate that nearly 80% of people in these regions rely on traditional medicines, predominantly herbal medicine, to meet their primary healthcare and socio-economic needs (Asafo-Agyei *et al.,* 2023). The therapeutic effects of these medicinal plants are diverse and multifaceted. One major mechanism is their antioxidant activity, which helps to mitigate oxidative stress, a factor that contributes to testicular damage (Moradi *et al.,* 2018).

### 1.2.1 ENTANDROPHAGMA UTILI

It is a large deciduous tree in the *meliaceae* family, reaching hights of approximately 45-60 meters with a straight bole up to 2 m in diameter (Orwa *et al.,* 2009). It is widely distributed across humid lowland rainforests of West and Central Africa, including Nigeria, Ghana, Cameroon, and the Democratic Republic of Congo (Keay, 1989). It is characterized by its extensive branch structure, short reddish-brown hairs clustered at the ends of its branchlets, and bark that is about 4 cm thick. Its seeds pods typically open while still on the tree. The tree features hairy flower clusters (tomentellous inflorescence) with white flowers and distinctive buttress roots (Tchouankev *et al.,* 1998).

***Plate. 1 Entandrophragma utili*** **(Orwa *et al.*, 2009)**

### 1.2.2 Taxonomical classification of E*ntadrophragma utili*

The taxonomical classification of E. utili according to Yakusu *et al.,* (2021) is as follows;

Kingdom: Plantae

Phylum (Division): Tracheophyta (Vascular plants)

Class: Magnoliopsida

Order: Sapindales

Family: Meliaceae

Genus: *Entandrophragma*

Species: *Entandrophragma utili*

### 1.2.3 Phytochemical Constituents

Several research has shown the presence of medically active substance, found in the stem bark of E*ntandrophragma utili* such as tetranotriter period called utilins lactone E*ntandrophagmin,* methyl

angolensate, heptan or triterperoid called entilins and ergosterol derivatives. The bark maceration is useful as tonic and stimulant (Muni *et al.,* 2008).

### 1.2.4 Biological Activities of E*ntandrophragma utili*

1. **Antioxidant Activity:** *Entandrophragma utili* bark and leaf extracts exhibit potent free radical scevenging and metal-chelating properties, attributed to high phenolic and flavonoid content (S. O. Usman, *et al.,* 2018).

2. **Anti-inflammatory Activity:** The anti-inflammatory potential of E*ntandrophragma utili* is primarily driven by its limonoid and flavonoid constituents, which target key inflammatory signalling pathway (Nguemfo *et al.,* 2022). The stem bark of *Entandrophragma utili* is used for the management of inflammation and pain, including conditions like rheumatism, arthritis and general swelling (Adeola *et al.,* 2023).

3. **Antimalarial activity:** Some compounds isolated from E*ntandrophragma utili* is used, specifically certain entilins (heptanor triterpenoids), have shown moderate in vitro antimalarial activity against chloroquine-resistant strains of Plasmodium falciparum (Vincent Ngouana, *et al.,* 2024).

4. **Anti sickling activity:** traditional healers in Nigeria and other parts of Africa use E*ntandrophragma utili* for the management of sickle cell disease (SCD). in vitro studies have validated this traditional us e, showing that extract of red blood cells in individuals with sickle cell disease, this is due to the presence of phytochemicals such as saponins, tannins and alkaloids which contributes to this anti sickling properties (O. E Adejumo *et al .,* 2011).

**Medicinal uses of E*ntandrophragma utili***

1. Sickle cell diseases (SCD)
2. Pain relief
3. Eye and ear infection

4. Antioxidant activity

5. Tonic and stimulant

6. Malaria

### 1.3 Carbon Tetrachloride-Mechanism of Testicular Toxicity

The molecular weight of carbon tetrachloride ($CCl_4$) is 153.82g/mole. It is a colorless, transparent, and volatile liquid. Carbon tetrachloride ($CCl_4$) is a well-established hepatotoxin and environmental pollutant used in experimental models to induce oxidative stress and organ damage. In the testes, $CCl_4$ causes toxicity through oxidative stress, disrupting spermatogenesis, steroidogenesis, and testicular histopathology.

#### 1.3.1 Mechanism of Testicular Toxicity:

#### 1.3.1 *Metabolic activation and reactive oxygen species (ROS) generation*

$CCl_4$ is metabolized by cytochrome P450 enzymes, primarily CYP2E1, into reactive *trichloromethyl* (-$CCl_3$) and *trichloromethyl peroxyl* (-$OOCCl_3$) radicals (Weber *et al.,* 2003). While the liver is the primary site of metabolism, testicular cells, including Leydig and Sertoli cells, express *CYP2E1,* enabling local bioactivation (Jiang *et al.,* 1998). These radicals initiate oxidative stress by attacking cellular components, increasing *ROS* levels by *2–3-fold* in rat testes (*1 mL/kg* $CCl_4$, intraperitoneal, 4 weeks) (Khan and Ahmed, 2015).

#### 1.3.2 Lipid peroxidation

The *–*$CCl_3$ *and –*$OOCCl_3$ radicals abstract hydrogen from polyunsaturated fatty acids in testicular cell membranes, triggering lipid peroxidation (Recknagel *et al.,* 1989). This produces malondialdehyde (*MDA*) and 4-hydroxynonenal, with *MDA* levels rising by 2–3-fold in $CCl_4$-treated rats (*1–2 mL/kg, 2–*

*4 weeks*) (*Al-Olayan et al., 2014)*. Testicular membranes, rich in lipids due to germ and Leydig cell composition, are particularly vulnerable, leading to disrupted membrane integrity and impaired spermatogenesis *(Khan et al., 2012).*

**1.3.3 Depletion of Antioxidant Defences***: $CCl_4$*-induced *ROS* overwhelm endogenous antioxidants, significantly reducing enzymatic and non-enzymatic defenses.

### *1.4* Oxidative Stress and Testicular Toxicity

Oxidative stress arises from an imbalance between free radicals and antioxidant defense mechanisms in the human body upon toxic exposure, ischemia/reperfusion, intensive exercise , chronic disease, aging and lifestyle that leads to several damages of tissues, cells and organs of the body (Mehmet and Celal, 2022).

Reactive oxygen species *(ROS)* and reactive nitrogen species *(RNS)* are endogenously generated as toxic byproducts of oxygen metabolism that is essential for living organisms. These free radicals consist of superoxide *($O_2^-$)*, hydrogen peroxide *($H_2O_2$),* nitric oxide *(NO),* hydroxyl radicals *(OH)*, peroxynitrite *($ONOO^-$)*, and lipid peroxyl radicals *(LOO)* ( Hong and Park, 2021). Free radical are reactive oxygen species which is continuously circulating through the body and also occur as a side effects of many metabolic reactions that take place in the human body (Adewoyin *et al.,* 2017).

Excessive *ROS* can attack lipids in sperm membranes, damage DNA, and impair mitochondrial function, all of which compromise sperm motility and viability (Agarwal *et al.,* 2006). Aitken and Roman (2008) emphasized that oxidative stress is a central mechanism in idiopathic male infertility, affecting both spermatogenesis and sperm function.

### 1.5 Antioxidant Defense Mechanism in the Testes

Antioxidants plays a vital role in counteracting oxidative stress. A complex antioxidant system comprising both antioxidant enzymes and non-enzymatic small molecular weight antioxidants has been developed in living system (Valko *et al.*, 2024). both enzymatic and non-enzymatic antioxidants exist in extracellular and intracellular environment to detoxify ROS.

#### 1.5.1 Enzymatic antioxidants

They neutralize reactive oxygen species *(ROS)* and prevent oxidative damage. The enzymatic antioxidants present in testes are superoxide dismustase *(SOD)*, catalase *(CAT),* glutathione peroxidase and reductase.

- **Superoxide Dismustase *(SOD)***

Superoxide dismutase is the frontline antioxidant enzyme that catalyzes the conversion of superoxide anion radicals ($O_2^-$) into hydrogen peroxide ($H_2O_2$) and oxygen. This prevents the accumulation of highly reactive superoxide radicals that can damage biomolecules, thereby preventing lipid peroxidation and improve sperm motility.

*SOD* isoforms *(Cu/Zn-SOD* in cytoplasm, *Mn-SOD* in mitochondria*)* are highly expressed in Leydig and Sertoli cells. *SOD* activity is critical for protecting spermatocytes, with levels *40–60%* higher in testes than in other tissues (Aitken and Roman, 2008).

- **Catalase *(CAT)***

Catalase primarily acts in peroxisomes, where it breaks down hydrogen peroxide into water and oxygen (Halliwell and Gutterridge, 2015). By eliminating hydrogen peroxide, *CAT* protects cells from oxidative stress and the generation of hydroxyl radicals *(-OH)*.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

- **Glutathione Peroxidase *(GPx)*:**

Glutathione peroxidase detoxifies hydrogen peroxide and lipid hydroperoxides into water and corresponding alcohols, using reduced glutathione (GSH) as a cofactor (Brigelius-Flohe and Maiorino, 2013). This is particularly important in protecting sperm membranes from lipid peroxidation (Khan *et al.,* 2012).

- **Glutathione Reductase *(GR):***

Glutathione reductase regenerates reduced glutathione (GSH) from GSSG using NADPH, maintaining GSH pools (Couto *et al.*, 2016). Glutathione reductase supports glutathione peroxidase (GPx) function, ensuring continuous *ROS* detoxification (Ojo *et al.,* 2017).

### 1.5.2 Non-Enzymatic Antioxidants

Non-enzymatic antioxidants also known as exogenous antioxidant, scavenge reactive oxygen species and support enzymatic systems. The non-enzymatic antioxidants present in the testes are reduced glutathione, vitamin E, vitamin C.

**Reduced glutathione (GSH)**

Reduced glutathione is a tripeptide and one of the most abundant non-enzymatic antioxidants in cells. It functions as a redox buffer, directly scavenging free radicals and serving as a substrate for GPx (Meister and Anderson, 1983). GSH is abundant in testicular cytosol, protecting germ and Leydig cells (Al-Olayan *et al.*, 2014). GSH also participates in detoxification reactions and helps maintain cellular redox homeostasis.

***Vitamin C:***

Vitamin C (ascorbic acid) is a water soluble antioxidant that scavenges superoxide and hydroxyl radicals and regenerate vitamin E (Padayatty *et al.,* 2003). High concentration in testicular interstitial fluid protect Leydig cells, while decrease in testicular vitamin C correlates with oxidative damage (Ojo *et al.*, 2017).

***Vitamin E (Tocopherol):***

Vitamin E is the principal lipid-soluble, chain-breaking antioxidant found in cell membranes, including the sperm plasma membrane which is rich in polyunsaturated fatty acids (PUFAs) (Azzi, 2018). It resides within the lipid bilayer and efficiently donates a hydrogen atom to lipid peroxyl radicals (LOO-), thereby interrupting the propagation phase of lipid peroxidation (Niki, 2014). This action protects the structural integrity and fluidity of sperm membranes, which is essential for sperm motility and acrosomal function (Aitken et al., 2022). Vitamin E works in concert with Vitamin C, which regenerates the tocopheroxyl radical of Vitamin E back to its active reduced form (Traber and Stevens, 2011).

# CHAPTER TWO

# 2.0 MATERIAL AND METHODS

## 2.1 Materials/Equipments Used

Water bath (Searchtech Instruments, England)

Analytical Weighing balance (S. METTLER)

Centrifuge 80-2 (Life Assistance Scientific, U.K)

Spectrophotometer 20D (Tekmel & Tekmel, USA)

Cotton wool (SAM SOLO GLOBAL RESOURCES, Nigeria)

Gloves (Pyrex, England)

Separation funnel

Syringe (HMA MEDICAL LIMITED, Nigeria)

Spatula (Pyrex, England)

Beakers (Pyrex, England)

Stirring rod (Pyrex, England)

Measuring cylinders

Test tubes (Pyrex, England)

Plain bottle (Pyrex, England)

Test tube rack (Pyrex, England)

Gavage

Refrigerator

PH meter (Pyrex, England)

Mortal and pistle

### 2.1.1 Reagents and Chemicals

All chemicals used were of good quality and of analytical grade. They were products of Pyrex, Nigeria. The reagents include:

Hydrochloric acid, sulphuric acid, sodium hydroxide, trichloroacetic acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate, hydrogen peroxide 30%, sodium carbonate, potassium hydroxide 85%, phosphate buffered saline *(pH: 7.4), sodium citrate, pyrogallol, disodium hydrogen phosphate, sodium hydrogen carbonate, EDTA-disodium, thiobarbituric acid, potassium permanganate.*

## 2.2 Collection of Plant Materials and Preparation

Fresh stem bark of *Entandrophragma utili* was obtained from the forest area in Ogun state and was identified by a taxonomist, Prof. H. Akinibosun of Plant Biology and Biotechnology Department, University of Benin. The stem bark was given a thorough wash and allowed to dry in a shaded area. The stem bark was pulvarized into small coarse particles using a mortar and pestle. The pulvarized extract was stored in a container until ready for use.

### 2.2.1 Extraction and Fractionation of plant material

The extraction process was carried out within the period of 72 hours, in which the pulvarized extract of *Entandrophragma utile* was soaked in a jar with methanol and stirred twice a day. Maceration method was used whereby a fermented mixture of *Entandrophragma utile* was poured into a sieve bag placed over a collection vessel (bowl). The mixture was again separated using the sieve bag, this process was repeated until enough herb residue was collected. The filtrate was placed in an open vessel to facilitate evaporation, prioritizing a higher solvent yield and less roughage. After drying, the filtrate was removed from the vessel, weighed and then grounded with a mortar and pestle with its total weight recorded.

### 2.2.2 Animals

Albino Wistar rats with an average weight of 120g-150g of male sex were selected for this study. The animals were obtained from the Animal House, Department of Anatomy, College of Basic Medical Science , University of Benin, Benin City, Edo State, Nigeria. The animals were kept in clean cages in a 12-hour light/dark cycle room with daily litter change. The animals were acclimatized for two (2) weeks before the experiment commenced. The animals were fed with rat chow and water ad libitum. The weights of the rats were monitored throughout the duration of the experiment. During the study, rats were maintained under standard condition and the ethics and experimental protocol governing the use and conduct of experiment with live animals were strictly observed as approved by the University of Benin's ethical committee on the use of laboratory animals for experimental purposes, as recommended by the National Research Council (US) Committee Guide for the Care and Use of Laboratory Animals (2011).

### 2.2.3 Experimental Design

Twenty-five (25) Wistar rats were divided into 5 groups of 5 rats each and treated as follows; *Entandrophragma utili* extract and fractions were administered orally for 28 days, while tetrachloromethane *($CCl_4$)* was induced intraperitoneally within the last 7 days of the experiment as shown below;

***Group I:*** (positive control); no *Entandrophragma utili* and *$CCl_4$* administered. Rats were given food and water ad libittum

***Group II:*** (Experimental control) rats were given a single dose of *$CCl_4$ (0.5 ml/kgbwt in olive oil 1:1; ip)* only.

***Group III:*** rats were treated with Crude Extracts *(400 mg/kgbwt)* daily for 28 days. After, rats were given a single dose of *$CCl_4$ (0.5 ml/kgbwt in olive oil 1:1; ip)* at the last seven days

***Group IV:*** rats were treated with Ethyl Acetate *(400 mg/kgbwt)* daily for 28 days. After, rats were given a single dose of *$CCl_4$ (0.5 ml/kgbwt in olive oil 1:1; ip)* in the last seven days of the experiment.

***Group V:*** rats were treated with Ethanol Residue *(400 mg/kgbwt)* daily for 28 days. After, rats were induced a single dose of *$CCl_4$ (0.5 ml/kgbwt in olive oil 1:1; ip)* in the last seven days of the experiment.

### 2.2.4 Animal Euthanization and Sample Collection

At the end of four weeks following the last treatment, the rats were fasted overnight and euthanized in a chloroform saturated chamber. Thereafter, they were ventrally dissected and the testes were excised and dried between layers of soft tissue paper and weighed.

### 2.2.5 Preparation Of Tissue Homogenates

Sample of blood and testes were obtained and used for biochemical and histopathological analysis. The blood sample collected were centrifuged at 3000 rpm for 10 mins and plasma were collected as the supernatant for biochemical analysis. The testes for histopathology was stored in plain bottles containing formalin and sent for histopathology.

## 2.3.0 BIOCHEMICAL ASSAYS

### 2.3.1 Estimation Of Glutathione Peroxidase (GPx) Activity

This was determined according to the method of Nyman (1959)

***Principle***

The principle is based on the auto-oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colouration which is read at *420 nm* on the spectrophotometer.

***Procedure***

A phosphate buffer of *1.25 mL, 1.25 mL* of $H_2O_2$, *0.75 mL* of water and *1.25 mL* of pyrogallol were added to *0.1 mL* of the sample . The reacting mixture was allowed to stand for 30 minutes at room temperature. A deep brown colour developed which was read with the spectrophotometer at *420 nm* at 0 s, 30 s, 60 s, and 90 s intervals.

### 2.3.2 MALONDIALDEHYDE

***Principle***

Malonaldehyde, $CH_2(CHO)_2$, is a product of lipid peroxidation. It reacts with thiobarituric acid *(TBA) t*o give a pink product which absorbs light at *535 nm*.

***Procedure***

*0.5 mL* volume of sample was added to a *1.0 mL* of equal volume of *TCA-TBA-HCL* solution and properly mixed. The solution was incubated for 15 minutes. At the end of the 15 minutes of incubation, the tubes were removed, allowed to cool. The flocculent precipitates formed during heating process were removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the supernatant was read at *535 nm* against the blank.

### 2.3.3 REDUCED GLUTATHIONE

Reduced glutathione (GSH) was determined by the Ellmans method (Ellmans, 1952).

***Principle***

The assay is based on the reaction of GSH with *5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB)* (also known as Ellman's reagent) that produces the TNB chromophore, which has a maximal absorbance at *412 nm.* The rate of formation of TNB, measured at 412 nm, is proportional to the concentration of GSH in the sample. The disulfide product *(GS–TNB)* is then reduced by Glutathione Reductase (GR) in the presence of NADPH, recycling GSH back into the reaction. Because GR reduces the Glutathione disulfide (GSSG) formed into 2 GSH, the amount of glutathione measured represents the sum of reduced and oxidized glutathione in the sample *([GSH]total = [GSH] + 2 × [GSSG]).* The rate of change in absorbance (Δ*A412 nm min–1)* is made to be linear for the convenience and consistency of measurement, and is linearly proportional to the total concentration of GSH. The concentration of an unknown sample is determined by calculating from the linear equation or the regression curve generated from several standards of GSH.

***Procedure***

To the tissue homogenates of *0.5 mL* volume, *1.25 mL of 10% TCA* was added, mixed and allowed to incubate for 5minutes at *230 C* and thereafter centrifuged at 3000 rpm for 10 minutes. Ellmans reagent *(0.25 mL )* and *1.5 mL* of phosphate buffer were added *to 0.5 mL* of supernatant, the content of the tube were mixed and absorbance of the samples was read at *412 nm* against the blank.

### 2.3.4 SUPEROXIDE DISMUTASE

***Principle:***

Superoxide dismutase is an indirect method which is based on the inhibitory effect of *SOD* in the initial rate of epinephrine auto-oxidation which is derived from the reaction proposed by Misra and Fridovich (1972), for the based catalyzed auto-oxidation of epinephrine.

***Procedure:***

Tissue homogenate *(0.4 mL)* was added to ice cold test tube containing *5 mL* of *0.05 M c*arbonate buffer *(pH 10.2) t*o which the reaction will start by the addition of 0.6 mL of freshly prepared 0.3mM epinephrine as the substrate to the buffered supernatant mixture which will be mixed by quickly by inversion while the blank or reference contain *5 mL of 0.05M* carbonate buffer *(pH 10.2), 0.4 mL* distilled water and *0.6 mL o*f freshly prepared *0.3 mM* epinephrine as the substrate. The increase in absorbance at 480 nm due to the adrenochrome formed will be monitored every 30 s for 120 s.

**2.3.5 CATALASE (CAT)**

***Principle:***

When $H_2O_2$ is generated in large quantity, Catalase is used for its removal. Estimation of Catalase will be based on the method of Cohen *et al.* (1970). Catalase is determined by measuring the rate of decomposition or disappearance of

hydrogen peroxide after the addition of the material containing the enzyme. It catalyzes the following reaction:

$H_2O_2 \rightarrow 2H_2O + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The product of hydrogen peroxide is measured by reacting it with excess potassium tetraoxomanganate (VII) and then, measuring the residual KMnO4 spectrophtometrically.

***Procedure****:*

Tissue homogenate *(0.5 mL)* was added to ice cold test tube and *0.5 mL* of distilled water was added into another ice cold tube as blank. The reaction was initiated by sequentially adding at fixed intervals *5 mL of cold 30 mM $H_2O_2$ Phosphate buffer (pH 7.4).* They were thoroughly mixed by inversion. After

exactly 3 minutes, the reaction was stopped sequentially at the same fixed interval by rapidly adding 1 mL of *6 M $H_2SO_4$* and mixed quickly by inversion. A volume of *7 mL of 0.01M* $KMnO_4$ was added to the test and blank tubes one at a time and was mixed twice by inversion. The absorbance was read at 480 nm within 30 to 60 seconds of mixing. The reference was prepared by adding 7 mL of 0.01M KMnO4 to a mixture of *5.5 mL phosphate buffer (pH 7.4) and 1 mL of 6 M $H_2SO_4$ solution.* The spectrophotometer was zeroed with the distilled water.

## 2.4 DATA ANALYSIS

The findings from this study was presented as (mean ± sd), and a statistical analysis was conducted to assess the significance of variant between the control group and the experimental group

## CHAPTER THREE

## 3.0 RESULT

### 3.1 Effect of crude extract and solvents fractions of *Entandrophragma utili* on superoxide dismustase (SOD) concentration in $CCl_4$ induced testes damage in Wistar rats

The effect of *E.utili* extract on superoxide dismustase concentration in $CCl_4$ induced testes damage in Wistar rats is presented in *Figure 3.1.* Administration of $CCl_4$ at doses of *(0.5 ml/kgbwt in olive oil 1:1; ip*) significantly *($p < 0.05$)* reduced the concentration of *SOD* when compared with normal control. Treatment with crude extract at doses of *(400mg/kgbwt)* daily for 28 days signigficantly *($p < 0.05$)* increased the concentration of *SOD* to a level comparable to the normal. However other plant solvents of *EU* (ethyl accetate and ethanol residue) showed non-significant *($p > 0.05$)* increase in SOD activity compared to the $CCl_4$ - only group, indicating a milder restorative effect.

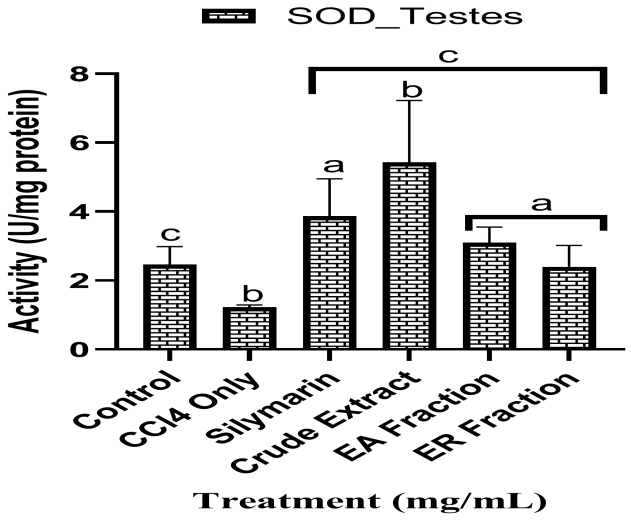


***Figure 3.1: Effect of Crude Extract and Fractions of EU on Superoxide dismutase Activity of the Testes of Carbon Tetrachloride-Intoxicated Wistar Rats.*** Values represent the mean ± Standard Error of Mean (n= 4). $CCl_4$: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, b = significant compared to the control group, c = significant compared to the $CCl_4$ group.

***.2 Effect of crude extract and solvents fractions of Entandrophragma utili on glutathione peroxidase (GPx) concentration in $CCl_4$ induced testes damage in Wistar rats***

The effect of ***E.utili*** extract on GPx concentration in $CCl_4$ induced testes damage in Wistar rats is presented in *Figure 3.2.* Administration of $CCl_4$ at doses of *(0.5 ml/kgbwt in olive oil 1:1; ip)* significantly *($p < 0.05$)* reduced the concentration of GPx at $CCl_4$ only group when compared with the normal control. post-treatment with *E.utili* extract at doses of *(400 mg/kgbwt)* daily for 28 days increased the activity of GPx, which was not statistically significant *($p > 0.05$)* compared to the $CCl_4$ group but demonstrated a clear trend towards normalization.

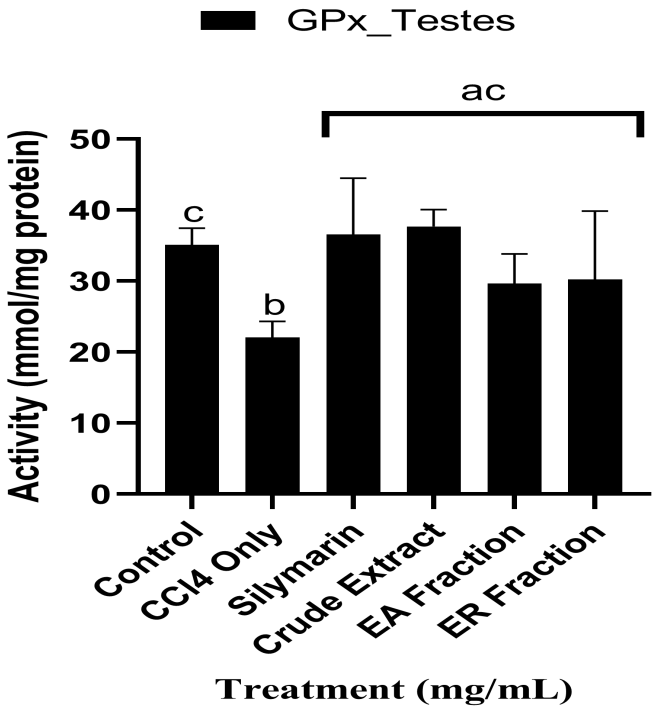


***Figure 3.2: Effect of Crude Extract and Fractions of EU on Glutathione Peroxidase Activity of the Testes of Carbon Tetrachloride-Intoxicated Wistar Rats.*** Values represent the mean ± Standard Error of Mean (n= 4). $CCl_4$: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant

compared to the control group, b = significant compared to the control group, c = significant compared to the $CCl_4$ group.

### 3.3 Effect of crude extract and solvents fractions of *Entandrophragma utili* on reduced glutathione (GSH) concentration in *$CCl_4$* induced testes damage in Wistar rats

The effect of *E.utili* extract on GSH concentration in $CCl_4$ induced testes damage in Wistar rats is presented in *Figure 3.3.* Administration of *$CCl_4$* at doses of (0.5 ml/kgbwt in olive oil 1:1; ip) non-significantly ($p > 0.05$) reduced the concentration of GSH when compared with normal control. Treatment with *E.utile solvents* at doses of (400 mg/kgbwt) daily for 28 days non-signigficantly ($p > 0.05$) increased the concentration of GSH when compared with normal control and *$CCl_4$. Evidently, the ethanol residue fraction appeared to have the most pronounced effect on GSH restoration among all treatments groups.*

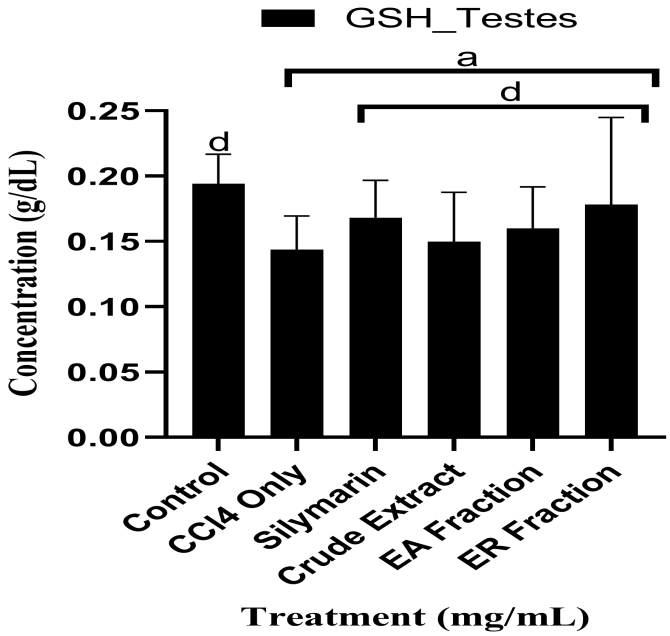


***Figure 3.3:*** **Effect of Crude Extract and Fractions of *EU* on Reduced Glutathione Concentration of the Testes of Carbon Tetrachloride-Intoxicated Wistar Rats.** Values represent the mean ± Standard Error of Mean (n= 4). $CCl_4$: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group d = non-significant compared to the $CCl_4$ group.

### 3.4 Effect of crude extract and solvents fractions of *Entandrophragma utili* on catalase *(CAT)* concentration in $CCl_4$ induced testes damage in Wistar rats

The effect of *E.utili* extract on GSH concentration in $CCl_4$ induced testes damage in Wistar rats is presented in *Figure 3.4.* Administration of $CCl_4$ at doses of (0.5 ml/kgbwt in olive oil 1:1; ip) non-significantly ($p > 0.05$) reduced the concentration of catalase when compared with normal control. Treatment with crude extract at doses of (400 mg/kgbwt) daily for 28 days significantly ($p < 0.05$) increased the concentration of catalase when compared with normal. However other plant fractions of *EU* (ethyl accetate and ethanol residue) showed no-significant ($p > 0.05$) increased when compared with the normal control.

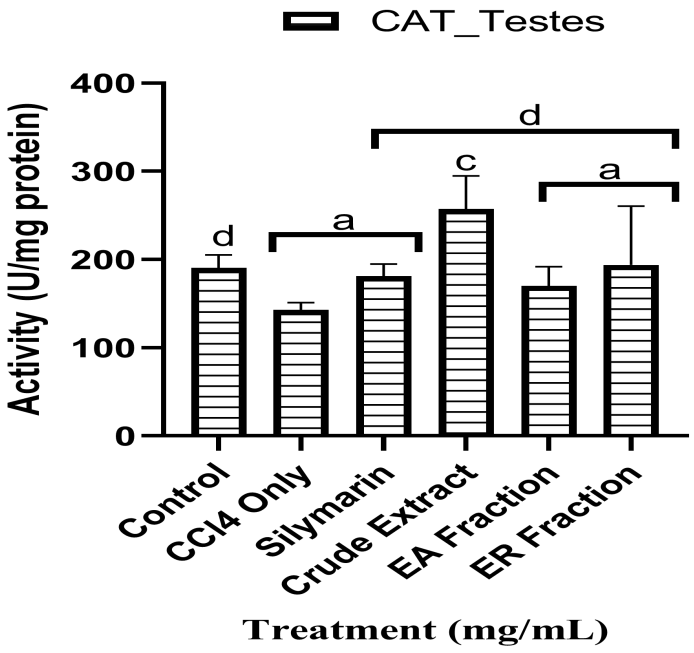


***Figure 3.4:*** **Effect of Crude Extract and Fractions of *EU* on Catalase Activity of the Testes of Carbon Tetrachloride-Intoxicated Wistar Rats.** Values represent the mean ± Standard Error of Mean (n= 4). $CCl_4$: Carbon tetrachloride, EA: Ethylacetate, ER: Ethanol residue. a = non-significant compared to the control group, c = significant compared to the $CCl_4$ group, d = non-significant compared to the $CCl_4$ group.

# CHAPTER FOUR

## DISCUSSION

This study was designed to evaluate the potential protective effect of *Entandrophragma utili* bark extract against carbon tetrachloride ($CCl_4$)-induced testicular oxidative stress in Wistar rats. The results demonstrate that $CCl_4$ intoxication significantly disrupts the testicular antioxidant defense system, while subsequent treatment with *E. utili* extract, particularly the crude extract, effectively mitigates this damage. The significant reduction in the activities of key antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) in the $CCl_4$-only group aligns with the established mechanism of $CCl_4$ toxicity.

Superoxide dismutase (SOD), is an antioxidant enzyme involved in the removal of oxygen species and free radicals. It is involved in the convertion of superoxide ions into oxygen and hydrogen peroxide thereby preventing lipid peroxidation and improve sperm motiliy. However, perturbations could make their activity lower than normal in the body. From the result in *figure 3.1*, it was evident that there were low levels of SOD activity in the testes of rats intoxicated with $CCl_4$ only. This indicates that $CCl_4$ was able to cause testicular damage. The observation aligns with the findings of Sonmez *et al. (*2006), who reported that carbon tetrachloride a known hepatoxin, is also toxic to reproductive organs inducing oxidative stress/damage by generating free radicals such as *trichloromethyl* and *peroxyl via cytochrome p450* metabolism. However treatments with the plant solvents showed an increase of SOD activity in the testes. The observed increase in SOD activity between the plant fractions and the crude extract was evident that the crude extract was

more active in the restorative potential of SOD activity in the testes when compared to the control.

Catalase (CAT), is an antioxidant enzyme involved in the decomposition of hydrogen peroxide to molecular oxygen and water thereby preventing the buildup of reactive oxygen species (ROS) that can damage DNA, proteins, and other cellular components. It is found in the peroxisome of living organisms. However, perturbations could make their activity lower than normal in the body. From the result in *figure* 3.4 it was observed that there were slightly low levels of catalase activity in the testes of rats intoxicated with $CCl_4$ only. This justifies the toxic potential of $CCl_4$ in reproductive organs with reduced disruption of catalase in the testes.

Treatments with the plant solvents showed an increase of catalase activity in the testes. However between the crude extract and the solvents, it was evident that the crude extract was more active and was able to reduce the assault in the testes, increasing catalase activity near that of the normal control. The observation aligns with the findings of Adebayo *et al.* (2020), who reported that accumulation of free radicals leads to increase in reactive oxygen species capable of attacking lipids in sperm membranes, damage DNA and impair mitochondrial function, all of which compromise sperm motility and viability.

Glutathione peroxidase (GPx) is an antioxidant enzyme that detoxifies hydrogen peroxide and lipid hydroperoxides into water and corresponding alcohols using reduced glutathione (GSH) as a cofactor (Brigelius-Flohe and Maiorino, 2013). However, perturbations could make their activity lower than normal in the body. From the result in *Figure* 3.2, it was evident that GPx activity in the testes of rats intoxicated with $CCl_4$

only were reduced when compared with the normal control, indicating that $CCl_4$ is able to induce testicular damage. Treatments with the plant solvents showed an increase of catalase activity in the testes. However between the crude extract and the plant solvents, it was evident that the crude extract was more active and was able to reduce the assault in the testes, increasing GPx activity near that of the normal control.

Reduced glutathione (GSH), is the active form of the antioxidant glutathione, which is naturally produced by every cells in the body. It functions as a redox buffer, directly scavenging free radicals and serving as a substrate for GPx. From the result in *Figure* 3.3, it was evident that GSH activity in the testes of rats intoxicated with $CCl_4$ only were slightly reduced when compared with the normal control, indicating that $CCl_4$ is able to induce testicular damage with less effect on GPx activity. However treatments with the plant solvents showed an increase of GSH activity in the testes. The observed increase in GSH activity between the plant solvents (ethyl acetate, ethanol residue) and the crude extract was evident that the plant solvents were more active in the restorative potential in the testes when compared to the normal control with ethanol residue showing greater restorative potential than ethyl acetate.

Taken together, these results demonstrate that *Entandrophragma utili* possesses significant restorative power, capable of restoring normal testes biochemical functions disrupted by $CCl_4$ intoxication. This bioactivity associated with this plant can be traced or linked to its phytochemical constituents.

## CONCLUSION

The findings from this study conclusively demonstrate that carbon tetrachloride ($CCl_4$) intoxication induces significant testicular oxidative stress in Wistar rats, as evidenced by the marked reduction in the activities of key antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). This oxidative assault disrupts the delicate redox balance within the testes, leading to cellular damage and compromised testicular function.

Conversely, the administration of *Entandrophragma utili* bark extract, particularly the crude extract, exhibited a remarkable protective and restorative effect. The extract significantly ameliorated the $CCl_4$-induced depletion of SOD, CAT, and GPx, effectively restoring their activities towards normal levels. This indicates that *E. utili* possesses potent antioxidant properties capable of counteracting the oxidative damage triggered by $CCl_4$. The superior efficacy of the crude extract over the solvent fractions suggests a synergistic interaction among its diverse phytochemical constituents, such as flavonoids, triterpenoids, and limonoids, which collectively contribute to its free radical-scavenging and antioxidant-enhancing capabilities.

Therefore, this study validates the traditional use of *Entandrophragma utili* and provides scientific basis for its potential application as a natural therapeutic agent against oxidative stress-induced male reproductive disorders.

## REFERENCE


Abarikwu, S. O., Akiri, O. F., Durojaiye, M. A., and Alabi, O. K. (2015). Reproductive toxicity of a glyphosate-based herbicide in male rats: the role of oxidative stress. *Journal of Toxicological Sciences*, **40**(5), 703–713.

Aitken, R.J., Roman, S.D. and Koppers, A.J. (2008). Mechanisms and consequences of oxidative stress in the male germ line. *Reproductive BioMedicine Online*, **14**(6), pp. 760–770.

Alrashdi, B.M., Al-Sagair, O.A., Al-Harbi, N.O., Alshammari, G.M., Alenazi, F.S., Alkhudhayri, L.S., Alruwaili, N.W., Alshammari, A.A. and Alshammari, M.A. (2025). Ameliorating effect of various fractions of Rumex hastatus roots against hepato- and testicular toxicity caused by CCl4. *Open Veterinary Journal*, **15**(1), pp. 402–415.

Amad, S.N., Ahmad, K.R.A., Usma, U.A., Fiza, F.M., Ali, R. & Amir-ud-din, U. (2022). Basella alba, Grewia asiatica, Solanum nigrum and Ficus carica fruit extracts ameliorate the testicular histopathology induced by $CCl_4$ exposure in albino mice: An experimental study. *International Journal of Reproductive BioMedicine*, **20**(3), pp. 195–206.

Assaf, J. C., Mortada, Z., Rezzoug, S.-A., Maache-Rezzoug, Z., Debs, E., & Louka, N. (2024). Comparative review on the production and purification of bioethanol from biomass: *A focus on corn. Processes*, **12**(5), 1001.

Astiz, M., de Alaniz, M. J., & Marra, C. A. (2009). Effect of pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. *Toxicology and Industrial Health*, **25**(1), 49–54.

Badr, G.M., Algefare, A.I. & Alfwuaires, M.A. (2021). Antioxidant potential of parsley leaf (Petroselinum crispum) essential oil on hypothyroidism and testicular injury in mice

intoxicated by carbon tetrachloride. BioMed Research International, 2021, Article 9989174.

Bayat, M., Koohpeyma, F., Montazeri-Najafabady, N., Dabbaghmanesh, M.H., Asmarian, N. and Hosseini, S.I. (2025). Protective effects of Silybum marianum seed extract on carbon tetrachloride-induced testicular toxicity in rats. *International Urology and Nephrology,* **57**(4), pp. 1123–1133.

Bello-Pérez, L.A., Flores-Silva, P.C. and Agama-Acevedo, E. (2019). Starch and its derivatives from maize: *From basic to industrial applications. Molecules*, **24**(11), 1–24.

Bello-Pérez, L.A., Flores-Silva, P.C. and Agama-Acevedo, E. (2019). Starch and its derivatives from maize: *From basic to industrial applications. Molecules,* **24**(11), 1–24.

Chen, L., R, P., P, M.K., Selvi, R.T., Ganesamoorthy, R., Sudan, S.J., Kasthuri, K. and Parameswari, R. (2025). Cadmium-induced testicular and spermatogonial damage ameliorated by Bryonia laciniosa Linn. seeds in Wistar rats: An animal study. *Biological Trace Element Research.* Advance online publication.

Elkirdasy, A., Hashem, K.S., Moustafa, G.G., Shokeir, H.A. and Ibrahim, A. (2021). Alpha-pinene alleviates $CCl_4$-induced renal and testicular injury in rats by targeting oxidative stress, inflammation, and apoptosis. *Chemico-Biological Interactions*, 350, Article 109695.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. Archives of Toxicology, **94**(3), pp. 651–715.

Gulcin, İ. (2025). Antioxidants: A comprehensive review. Archives of Toxicology, 99(5), pp. 1893–1997.

Hashem, A.S., Mohamed, A.A., El-Sayed, Y.S., El-Sayed, S.A. and Ali, H.A. (2024). Protective effect of curcumin on testicular damage caused by carbon tetrachloride exposure in rats. Reproduction, Fertility and Development, **36**(12), pp. 1789–1799.

Ibrahim, A.M., El-Sayed, Y.S., Al-Jabr, H.A., El-Sayed, S.A. and Hashem, K.S. (2022). Chlorella vulgaris ameliorates testicular toxicity induced by carbon tetrachloride in male rats via modulating oxidative stress. Andrologia, 2022, Article 14495.

Khalil, S.R., Abdel-Daim, M.M., Abd El-Hamid, H.S., El-Aziz, R.M., Al-Humiany, A.A., Mansour, M.A. & El-Mandouh, Z.A. (2021). The ameliorative effects of Lactobacillucoagulans and Lactobacillus casei probiotics on $CCl_4$-induced testicular toxicity based on biochemical, *histological and molecular analyses in rat*. Andrologia, **53**(2), Article e13894.

Mahmoud, A.M., Abd El-Tawab, S.M., Abdel-Rahman, M.H., Youssef, M.M., Ahmed, A.A. and Abdel-Aziz, M.A. (2024). Sophora pachycarpa root extract has ameliorative effects on testicular injury but is hepatotoxic in carbon tetrachloride intoxicated rats. *Journal of Medicinal Plants and By-products*, **13**(2), pp. 189–200.

Mokale Kognou, A.L., Kopa Kowa, T., Pateriya, P., Pal, P.N., Mouokeu, R.S., Tchinda Tiabou, A., Agbor Agbor, G., Pawar Singh, R. and Ngono Ngane, R.A. (2020). Pharmacological evidence of Vitex thyrsiflora, Entandrophragma cylindricum, and Anonidium mannii used for the management of inflammation in Cameroon. *Journal of Basic and Clinical Physiology and Pharmacology*, **31**(6), 20190053.

Ngouana, V.R., Tchatat, M.H.T., Tsouh, V.F.P.N., Tsafack, E.G., Tazoho, W.A., Tchatat, B.M.T., Tchatat, M.H.T. & Tsouh, V.F.P.N. (2025). Exploring the antimalarial potential of

Entandrophragma utile and Melochia umbellata extracts. *Chemistry & Biodiversity,* **22**(1), Article e202401314.

Othman, M.S., Afify, M., El-Sayed, Y.S., Ibrahim, A.M. and Abdel Moneim, A.E. (2023). Comparing the effects of Cyperus esculentus hydroethanolic extract and Euterpe oleracea on reproductive efficacy against cadmium-induced testicular toxicity in male rats. Heliyon, **10**(3), Article e25123.

Othman, M.S., Afify, M., El-Sayed, Y.S., Ibrahim, A.M. and Abdel Moneim, A.E. (2023). Comparing the effects of Cyperus esculentus hydroethanolic extract and Euterpe oleracea on reproductive efficacy against cadmium-induced testicular toxicity in male rats. Heliyon, **10**(3), Article e25123.

Perrone, P., De Rosa, C. and D'Angelo, S. (2023). Antioxidants and phytochemicals—A scoping review for Nordic Nutrition Recommendations 2023. *Scandinavian Journal of Public Health*, **51**(8), pp. 1170–1180.

Ranjbar, M., Shariati, M., Asgari, H.R., Ahmadvand, H., Amini, M. and Asgari, M. (2022). Protective effects of Teucrium polium on carbon tetrachloride-induced testicular toxicity in rats. Andrologia, 2022, Article 14567.

Ranjbar, M., Shariati, M., Asgari, H.R., Ahmadvand, H., Amini, M. and Asgari, M. (2022). Protective effects of Teucrium polium on carbon tetrachloride-induced testicular toxicity in rats. Andrologia, 2022, Article 14567.

Riaz, M., Rahman, N.U., Zia-Ul-Haq, M., Jaffry, T. and Manea, R. (2023). Phytobioactive compounds as therapeutic agents for human diseases: *A review. Food Science & Nutrition,* **11**(7), pp. 3308–3322.

Samad, A., Ijaz, M.U., Ashraf, A., Sajid, M., Imran, M. and Abbas, K. (2020). Methanolic extract of Nepeta paulsenii as an ameliorative agent against $CCl_4$ induced testicular damage in male albino rats. *Journal of King Saud University - Science*, **32**(3), pp. 1168–1174.

Sultana, S., Khan, H., Perveen, S. and Alomar, S.Y. (2021). Defensive impact of propolis against $CCl_4$ actuated rats' testicular damage. Oxidative Medicine and Cellular Longevity, 2021, Article 6674186.

Zafar, R., Khan, M.A., Islam, M.U. and Rauf, A. (2022). In vivo antioxidant efficacy and therapeutic potential of Artemisia brevifolia leaves extract against $CCl_4$-induced reproductive damages in male albino rats. *Saudi Pharmaceutical Journal,* **30**(2), pp. 193–203.

Zahedi, A., Dehghan, M.H., Razi, M., Malekinejad, H. and Rezaei, A. (2021). Sophora pachycarpa root extract improves testicular damage in carbon-tetrachloride intoxicated rats. Zahedan *Journal of Research in Medical Sciences*, **23**(4), Article e64950.

# APPENDIX

## *Preparation of Reagents*

*Normal saline (0.9%)*

Sodium Chloride (9 grams) was measured and dissolved in 200 ml of distilled water. The solution was then brought up to 1000 ml mark in a measuring cylinder by adding more distilled water.

Phosphate Buffer (0.05 m, pH 7.4)

Dissolved 0.34 ml of 30% $H_2O_2$ in 20 ml phosphate buffer and make up to 100 ml

*Epinephrine*

Dissolved 5 mg of epinephrine in 1000 ml 0.05 M of HCL

Trichloroacetic Acid

Dissolved 10g in 100 ml of distilled water ($DH_20$).

*Ellmas Reagent*

Sodium Citrate 2g plus DTNB 0.0189 in 100 ml of distilled water.

*Potassium Permanganate (0.01M)*

0.158 mg of potassium permanganate was dissolved in 100 ml of distilled water.

*Sulphuric acid*

Dissolved 163.2 mg of sulphuric acid in 600ml of distilled water to make up to 1000 ml of distilled water.

*Hydrochloric acid*

Concentrated Hydrochloric acid was added to 50ml of distilled water and then made up to 100ml using distilled water.

*Carbonate buffer (0.05 m, pH 10.2)*

Sodium Carbonate (2.014g), Sodium Bicarbonate (2.604), EDTA (0.372g) where dissolved in 900 ml of distilled water. The pH was then raised to 10.2 using Hydrochloric acid. The volume was adjusted to 100ml by adding more distilled water.

## CALCULATIONS

**Superoxide dismutase activity:**

$$\% \text{ inhibition} = \frac{(OD\ test - OD\ ref)\ X\ \ 100}{OD\ test}$$

$$\text{SOD activity} = \frac{inhibition}{5\%\ x\ \ Y}$$

Where Y = mg of protein in the volume of the sample used.

**Catalase activity:**

$$\text{Enzyme activity (Units/L)} = \frac{(\Delta\ Abs\ x\ Total\ assay\ volume)}{\Delta\ t\ \ x\ \mathcal{E}\ x\ l\ x\ Enzyme\ \ Sample\ volume}$$

Where $\Delta\ t$ is the time of incubation (minutes),

$\Delta\ Abs$ is the change in absorbance,

$\mathcal{E}$ is the extinction coefficient of substrate in units of 0.36M/cm

l is the cuvette diameter (1 cm).

**Glutathione peroxidase activity:**

The activity of GPx was calculated as:

Enzyme activity (U/L) = 8412 x Abs 340 nm/min

**Reduced glutathione:**

The concentrations of reduced glutathione was determined using the formula

$$GSH = \frac{}{E\ XL\ X\ df}$$

Where df = dilution factor

Molar ext. coefficient = 13.6,

L= 1

**ABBREVIATIONS**

EU- *Entandrophragma utile*

$CCl_4$ - carbon tetrachloride

SEM – Standard Error of the mean

SOD- Superoxide dismutase

CAT- Catalase

GPx- Glutathione peroxidase

GSH- Reduced glutathione

H – Head

B – Back

T – Tail

ABD – Abdomen

FL – Fore limb

HL – Hind limb

S – Stomach

$A_{sample}$ – Absorbance of sample

$A_{std}$ – Absorbance of standard

## PLANT EXTRACT ADMINISTRATION

### CRUDE EXTRACT

| RAT LABEL | WEIGHT | DOSE (mg) | VOLUME |
|---|---|---|---|
| **H** | **193.71** | **77.48** | **0.77** |
| **T** | **194.64** | **77.86** | **0.78** |
| **ABD** | **174.15** | **69.66** | **0.70** |
| **S** | **198.45** | **79.38** | **0.79** |
| **HL** | **200.57** | **80.23** | **0.80** |
| **B** | **171.15** | **68.46** | **0.68** |
| **FL** | **186.45** | **74.58** | **0.75** |

**ETHANOL RESIDUE**

| RAT LABEL | WEIGHT | DOSE (mg) | VOLUME |
|---|---|---|---|
| H | 153.16 | 61.26 | 0.61 |
| T | 168.08 | 67.23 | 0.67 |
| ABD | 175.28 | 70.11 | 0.70 |
| S | 183.77 | 73.51 | 0.70 |
| HL | 166.13 | 66.45 | 0.74 |
| B | 156.61 | 62.64 | 0.66 |
| FL | 123.91 | 49.56 | 0.63 |

**ETHYL ACETATE**

| RAT LABEL | WEIGHT | DOSE (mg) | VOLUME |
|---|---|---|---|
| H | 168.83 | 67.5 | 0.68 |
| T | 144.73 | 57.89 | 0.58 |
| ABD/FL | 157.83 | 63.13 | 0.63 |
| S | 146.01 | 68.40 | 0.58 |
| HL | 197.78 | 79.11 | 0.79 |
| B | 178.38 | 71.35 | 0.71 |
| FL | 183.22 | 73.29 | 0.73 |