ANTIOXIDANT STATUS OF THE LIVER AND KIDNEY OF WISTAR RAT TREATED WITH ETHANOL EXTRACT OF *Funtumia elastica*

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ANTIOXIDANT CAPACITY OF THE LIVER AND KIDNEY OF WISTAR RAT WITH ETHANOL EXTRACT OF *FUNTUMIA ELASTICA*

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A STUDY PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN.

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CERTIFICATION

I, hereby certify that this project was carried out by CHIMA AKACHI VICTOR with matriculation number LSC1906432, in the department of Biochemistry, faculty of Life Sciences, University of Benin, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) Honours degree.

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DEDICATION

This project is dedicated to God Almighty, the giver of life and wisdom. I also dedicate it to my Mother, Mrs. Chima Mabel for her love, encouragement and financial support. My siblings Goodness Chima, Noble Chima and my friend Akhilome Ahmed

I also dedicate it to all those out there who did not have the opportunity to attend a higher citadel of learning to gather such knowledge.

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My profound and utmost gratitude to God almighty, for His mercies and grace He bestowed on me throughout my academic journey in this university.

My deepest gratitude to my wonderful supervisor: Dr. O. C.Ugbeni for his love, time and attention. May God bless you.

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My greatest gratitude to my Mother for her love and support all through my time as a student, the Lord continually bless you. mnbAnd to my Uncle Mr. Paul Alelumhe

To everyone else who has in one way or another influenced the success of this study, I am eternally grateful.

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ABSTRACT

This report investigates the antioxidant capacity of an ethanol extract from the plant Funtumia elastica on the liver and kidneys of Wistar rats. Antioxidants play a crucial role in protecting cells from oxidative stress, which can lead to various diseases. The study evaluated the effects of the plant extract on several antioxidant parameters, including malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) activity in the liver and kidney tissues.

The results demonstrated that the ethanol extract modulated these antioxidant parameters to varying degrees in the liver and kidneys. Notably, the extract exhibited a protective effect against lipid peroxidation in the liver, as evidenced by significantly reduced MDA levels in Groups 3 and 4 compared to the control. Additionally, catalase activity was significantly upregulated in the liver of Group 2. However, GSH levels were significantly lower in the livers of treated groups compared to controls, suggesting potential compromise of the antioxidant defense system. No significant differences were observed for SOD and GPX activities in both organs across the groups.

While the extract showed promising antioxidant effects, particularly in the liver, further research is needed to elucidate the underlying mechanisms and explore potential therapeutic applications. The findings contribute to the understanding of Funtumia elastica's antioxidant properties and its potential use in managing oxidative stress-related conditions affecting vital organs.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 Background of Study

The antioxidant capacity of the ethanol extract of *Funtuma elastica*, a plant species, on the liver and kidney of Wistar rats is an interesting research topic. Antioxidants play a crucial role in protecting cells from oxidative stress, which can lead to various diseases and dysfunctions. The study of plant extracts and their potential antioxidant properties is an area of interest in natural product research and pharmacology.

Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, plays a crucial role in the pathogenesis of various diseases and complications (Sies, 1997). The liver and kidneys are vital organs that are particularly vulnerable to oxidative damage due to their high metabolic activity and exposure to environmental toxins (Cederbaum *et al.,* 2009; Vaisharru *et al.,* 2022; Menezes *et al.,* 2022).

The liver is the primary organ responsible for detoxification and metabolic processes, making it susceptible to oxidative stress-induced damage, which can lead to conditions such as non-alcoholic fatty liver disease (NAFLD) and hepatotoxicity (Masarone *et al.,* 2018). Similarly, the kidneys are essential for filtering waste products and maintaining fluid balance, and oxidative

stress can contribute to the progression of chronic kidney disease (CKD) and renal dysfunction (Small *et al*., 2012).

In recent years, there has been a growing interest in exploring natural sources of antioxidants, particularly from medicinal plants, as they are believed to be safer and more biocompatible compared to synthetic antioxidants (Krishnaiah *et al*., 2011). Funtumia elastica, commonly known as the Lagos rubber tree, is a plant species native to West and Central Africa that has been traditionally used for various medicinal purposes. Preliminary studies have suggested that extracts from this plant possess antioxidant properties due to the presence of phenolic compounds, flavonoids, and other phytochemicals (Adedayo *et al.,* 2015; Olubomehin *et al.,* 2013).

However, the potential antioxidant capacity of Funtumia elastica extract on vital organs, such as the liver and kidneys, has not been extensively investigated. Therefore, this study aims to evaluate the antioxidant effects of the ethanol extract of Funtumia elastica on these organs in an in vivo model using Wistar rats.

By investigating the antioxidant capacity of this plant extract and its potential protective effects against oxidative damage in these crucial organs, this research may contribute to the development of novel therapeutic strategies or preventive measures for oxidative stress-related diseases and complications (Lobo *et al.,* 2010). Additionally, this study may provide insights into the mechanisms underlying the antioxidant activity of Funtumia elastica and its potential applications in promoting overall health and well-being (Saikia and Loken, 2010).

1.1.2 Justification or Study

Oxidative stress is a significant contributing factor to various pathological conditions, including liver diseases and kidney disorders (Sies, 1997; Cederbaum *et al*., 2009; Vaisharru *et al*., 2022; Menezes *et al*., 2022). The liver and kidneys are vital organs that are particularly susceptible to oxidative damage due to their high metabolic activity and exposure to environmental toxins. Prolonged oxidative stress in these organs can lead to severe complications, such as non-alcoholic fatty liver disease (NAFLD), hepatotoxicity, chronic kidney disease (CKD), renal dysfunction, impaired fertility, and hormonal imbalances (Masarone *et al*., 2018; Small *et al.,* 2012; Menezes *et al*., 2022; Agarwal *et al.,* 2014).

While synthetic antioxidants are available, there is a growing interest in exploring natural sources of antioxidants, such as those derived from medicinal plants, due to their perceived safety and biocompatibility (Krishnaiah *et al.,* 2011). *Funtumia elastica*, a plant species native to West and Central Africa, has been traditionally used for various medicinal purposes and preliminary studies have suggested that its extracts possess antioxidant properties owing to the presence of phenolic compounds, flavonoids, and other phytochemicals (Adedayo *et al*., 2015; Olubomehin *et al*., 2013).

However, the potential antioxidant capacity of *Funtumia elastica* extract on vital organs, particularly the liver and kidneys, has not been extensively investigated. Evaluating the antioxidant effects of this plant extract on these organs is crucial as it may provide insights into potential therapeutic strategies or preventive measures for oxidative stress-related diseases and complications affecting these organs (Lobo *et al*., 2010).

By conducting an in vivo study using Wistar rats as an animal model, researchers can investigate the antioxidant capacity of *Funtumia elastica* ethanol extract on the liver, kidneys, and testis. This study can assess various parameters, such as antioxidant enzyme activities, oxidative stress markers, histological changes, and organ-specific functional parameters, to determine the potential protective effects of the extract against oxidative damage (Saikia and Loken, 2010).

Furthermore, understanding the mechanisms underlying the antioxidant activity of *Funtumia elastica* extract may contribute to the development of novel therapeutic approaches or nutraceutical products for promoting overall health and well-being.

Given the significant burden of oxidative stress-related diseases and the need for safe and effective antioxidant interventions, this study is justified in exploring the potential antioxidant capacity of *Funtumia elastica* extract on vital organs and its potential applications in promoting better health outcomes.

1.1.3 Aim of the Study

The aim of this research is to determine the Antioxidant capacity of ethanol extract of *Funtumia elastica* on the liver and kidney of wistar rat with

1.1.4 Objective of the Study

The objective of this research is to access the levels of the following parameters when administered with *F. elastica*:

- Superoxide dismutase
- Reduced glutathione

- Catalase
- Malondialdehyde
- Glutathione peroxidase

1.2 Literature review

1.2.1 Introduction

Nature has long been a rich source of medicinal compounds, and various plant species have been explored for their potential therapeutic properties (Rates, 2001). Among these is *Funtumia elastica*, a tree species native to West Africa, which has been used in traditional medicine for treating various ailments. The bark, leaves, and roots of this plant have been employed in folklore remedies for conditions such as fever, malaria, and respiratory disorders (Adjanohoun *et al.,* 1988).

In recent years, there has been growing interest in investigating the potential health benefits of plant extracts, particularly their antioxidant properties (Gulcin *et al*., 2020). Antioxidants play a crucial role in neutralizing free radicals and mitigating oxidative stress, which can lead to cellular damage and contribute to the development of various diseases (Lobo *et al*., 2010). The body's natural antioxidant defense mechanisms can be overwhelmed by excessive free radical production, making it essential to explore external sources of antioxidants (Sies, 2018).

The liver and kidney are vital organs that are particularly vulnerable to oxidative stress, which can impair their functions and potentially lead to adverse health consequences (Birben *et al.,* 2012). Oxidative stress in the liver can disrupt metabolic processes, contributing to liver diseases

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such as hepatitis and cirrhosis (Cichoż-Lach and Michalak, 2014). In the kidneys, oxidative damage can compromise their filtering and excretory functions, leading to the development of kidney disorders (Reznikov *et al.,* 2019). Additionally, oxidative stress in the testis has been linked to male infertility and reproductive dysfunction (Aitken and Curry, 2011).

In this context, exploring the antioxidant capacity of natural plant extracts, such as those derived from *Funtumia elastica,* holds significant promise. By identifying and harnessing the antioxidant potential of these extracts, researchers aim to develop potential therapeutic strategies to combat oxidative stress and promote overall organ health (Zhang *et al*., 2015).

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1.2.2 Overview of *Funtumia elastica* and its traditional uses

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Funtumia elastica, also known as the rubber vine or rubber tree, is a plant species belonging to the Apocynaceae family. It is a large tree found primarily in the tropical regions of West Africa, particularly in countries like Nigeria, Côte d'Ivoire, and Ghana (Anoliefo and Ikhajiagbe, 2012). The plant has a long history of traditional use among various ethnic groups in these regions.

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In traditional medicine systems, different parts of Funtumia elastica have been employed for their therapeutic properties. The bark has been widely used as a remedy for various ailments, including fever, malaria, respiratory infections, and diarrhea (Adjanohoun *et al.,* 1988; Betti, 2004). Additionally, the bark has been applied topically for the treatment of skin diseases and as a dressing for wounds and sores, owing to its believed antiseptic and healing properties (Burkill, 1985).

The leaves of *Funtumia elastica* have also been utilized in traditional practices. Leaf decoctions have been consumed as a treatment for cough, bronchitis, and other respiratory conditions (Neuwinger, 2000). Furthermore, the leaves have been used in traditional birth practices, with some communities using them as a means of inducing labor or facilitating childbirth (Betti, 2004).

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Beyond its medicinal applications, *Funtumia elastica* has also been valued for its economic significance. The plant is a source of latex, which has been exploited for the production of rubber (Burkill, 1985). This latex has been used in the manufacture of various rubber products, contributing to the local economies of the regions where the plant is found.

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The widespread traditional use of *Funtumia elastica* has sparked scientific interest in investigating its phytochemical composition and potential therapeutic properties. Researchers have begun to explore the plant's extracts for their biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties (Adeniyi *et al*., 2016; Oyebode et al., 2020).

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1.2.3 Importance of Antioxidants and their Role in Maintaining Organ Health

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Antioxidants play a vital role in maintaining overall health and preventing various diseases. They are substances that neutralize free radicals, which are highly reactive molecules produced during normal metabolic processes or as a result of exposure to environmental factors such as pollution, radiation, and tobacco smoke (Lobo *et al.,* 2010). Free radicals can cause oxidative stress by damaging cellular components like proteins, lipids, and DNA, leading to cell dysfunction and tissue damage (Sies, 1997).

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The human body has an intricate antioxidant defense system that includes enzymes like superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants like vitamins C and E, carotenoids, and flavonoids (Birben *et al.,* 2012). However, this natural defense system can be overwhelmed by excessive free radical production, leading to an imbalance and increased oxidative stress, which has been implicated in the pathogenesis of various diseases, including cardiovascular disorders, neurodegenerative diseases, cancer, and aging (Sies, 2018).

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Maintaining organ health is crucial for overall well-being, and oxidative stress can have detrimental effects on vital organs like the liver, kidneys, and testis... (Continues with information on the effects of oxidative stress on these organs).

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1.2.4 Significance of studying the antioxidant capacity of plant extracts

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Studying the antioxidant capacity of plant extracts is significant for several reasons:

1. Plant extracts are rich sources of bioactive compounds with antioxidant properties, including polyphenols, flavonoids, and carotenoids (Gulcin *et a*l., 2020).

2. Exploring the antioxidant potential of plant extracts can lead to the development of natural antioxidant therapies and nutraceuticals for the prevention and management of oxidative stress-related diseases (Burruel *et al.,* 2020).

3. Understanding the mechanisms of action and specific bioactive compounds responsible for the antioxidant effects can aid in the development of targeted therapeutic interventions (Zhang *et al.,* 2015).

4. Studying the antioxidant capacity of plant extracts on specific organs can provide insights into their potential protective effects and potential applications in organ-specific diseases (Reznikov *et al*., 2019).

By investigating the antioxidant capacity of plant extracts like those derived from *Funtumia elastica,* researchers can contribute to the development of novel and effective antioxidant therapies, ultimately promoting better organ health and overall well-being.

1.2.5 Mechanisms of action and potential bioactive compounds in *Funtumia elastica* extract

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Funtumia elastica is a plant species that has been used in traditional medicine for various purposes, including the treatment of fever, malaria, and respiratory disorders (Adjanohoun *et al*., 1988). Recent studies have begun to investigate the phytochemical composition and biological activities of extracts derived from different parts of this plant. Preliminary research has identified the presence of bioactive compounds, such as flavonoids, tannins, and terpenoids, in the extracts of *Funtumia elastica* (Adeniyi *et al.,* 2016; Oyebode *et a*l., 2020). These compounds are known to possess antioxidant properties and may contribute to the potential hepatoprotective effects of the plant extract.

While the specific mechanisms of action and bioactive compounds responsible for the antioxidant capacity of Funtumia elastica extract are yet to be fully elucidated, some proposed mechanisms include free radical scavenging, inhibition of lipid peroxidation, and modulation of antioxidant enzyme activities (Burruel *et al.,* 2020). Further research is needed to characterize the phytochemical profile, identify the major antioxidant constituents, and understand their molecular targets and modes of action in protecting the liver from oxidative stress.

Findings of some studies have also revealed the potential nephroprotective properties of *Funtumia elastica* extract. While there is limited research specifically investigating the effects of *Funtumia elastica* extract on kidney health, some studies have explored its potential antioxidant and nephroprotective properties. Adeniyi *et al*. (2016) evaluated the antioxidant activity of *Funtumia elastica* leaf extract using various in vitro assays and found that the extract exhibited significant free radical scavenging and reducing power, suggesting its potential as a natural antioxidant source.

In another study, Oyebode *et al*. (2020) investigated the nephroprotective effects of *Funtumia elastica* stem bark extract in a rat model of gentamicin-induced nephrotoxicity. They found that the extract attenuated gentamicin-induced renal injury, as evidenced by reduced serum creatinine and urea levels, improved glomerular and tubular architecture, and decreased oxidative stress markers in the kidney tissues. These findings suggest that the antioxidant properties of *Funtumia elastica* extract may contribute to its nephroprotective effects.

However, further research is needed to fully elucidate the specific mechanisms of action and bioactive compounds responsible for the potential nephroprotective effects of Funtumia elastica extract. Additionally, more in vivo studies are required to evaluate the efficacy and safety of the extract in various kidney disease models and to investigate its potential for clinical applications in the prevention and management of renal disorders.

1.2.6 The liver

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The liver is the largest internal organ in the human body, weighing approximately 1.6 kg in adults. It is located in the upper right portion of the abdominal cavity, below the diaphragm. It

receives blood supply from the portal vein and hepatic artery. It is made up of hepatocytes arranged into liver lobules. Also contains other cell types like Kupffer cells, stellate cells.

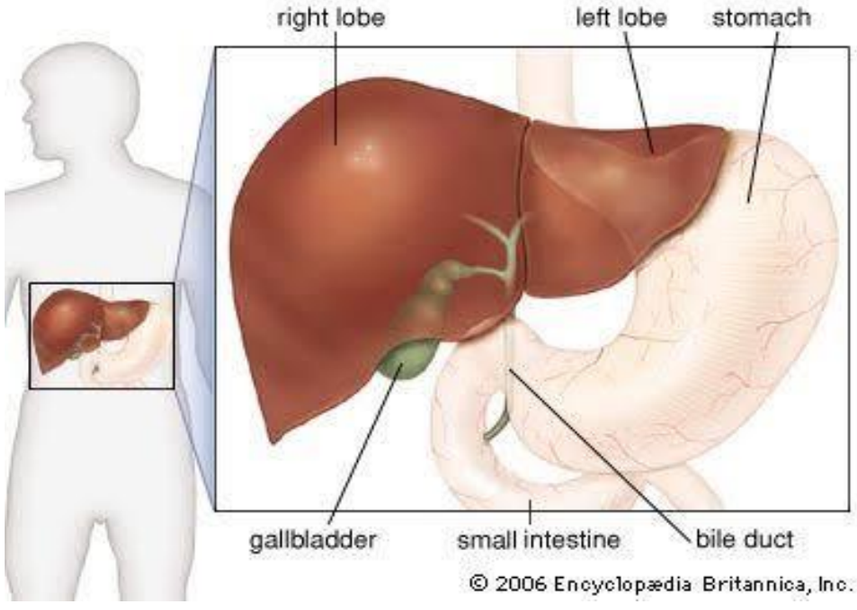


Figure 2 Liver (Feng *et al.*, 2017)

Therefore, the liver has diverse, vital functions in metabolizing nutrients, detoxification, synthesis, and filtering blood. Essential roles of the liver include:

- Metabolism of carbohydrates, proteins, fats, hormones, medications
- Detoxification and excretion of drugs, alcohol and environmental toxins
- Bile production for fat digestion and excretion
- Storage of vitamins, minerals, glycogen
- Blood filtration to remove bacteria, old blood cells
- Synthesis of blood clotting factors, amino acids, glucose
- Activation of digestive enzymes from the pancreas

1.2.6.1 Oxidative stress and its impact on liver function

Oxidative stress is a state of imbalance between the production of free radicals and the body's antioxidant defense mechanisms (Sies, 1997). In the liver, excessive free radical generation can occur due to various factors, including exposure to toxins, alcohol consumption, and metabolic disorders (Cichoż-Lach and Michalak, 2014). These free radicals can damage liver cells, leading to inflammation, fibrosis, and ultimately, liver dysfunction (Masalkar and Abhang, 2005). Oxidative stress has been implicated in the pathogenesis of various liver diseases, such as non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, viral hepatitis, and liver cancer (Zhang *et al*., 2020).

1.2.6.2 Antioxidant Capacity and the Liver

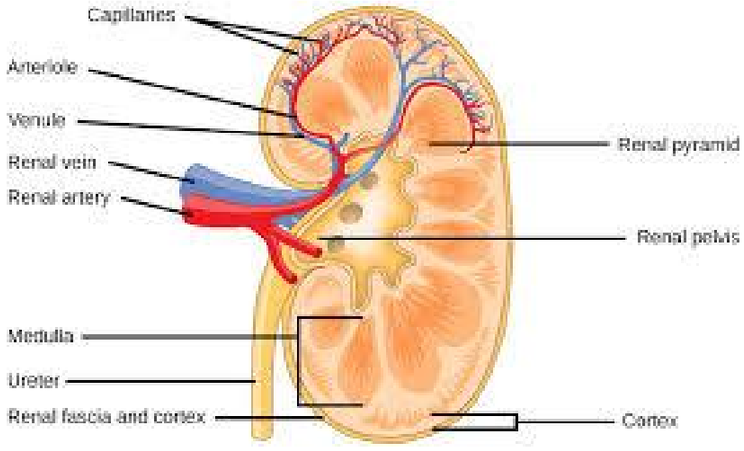
The liver plays a crucial role in maintaining overall health by performing various metabolic functions, including detoxification, protein synthesis, and bile production. However, the liver is susceptible to oxidative stress, which can lead to cellular damage and impaired liver function (Cichoż-Lach and Michalak, 2014). Antioxidants play a vital role in protecting the liver from oxidative stress by scavenging free radicals and preventing lipid peroxidation (Moreno et al., 2017). Studies have shown that plant extracts with high antioxidant capacity can potentially ameliorate liver injury and improve liver function (Ateyya *et al*., 2019).

Numerous studies have explored the potential of plant extracts as natural antioxidants and their protective effects on the liver. For instance, research has shown that extracts from green tea

(Camellia sinensis) possess significant antioxidant activity and can protect against liver injury induced by various hepatotoxic agents (Singal *et al*., 2001). Similarly, extracts from turmeric (Curcuma longa) have demonstrated antioxidant and hepatoprotective properties, attributed to their bioactive compound curcumin (Girish and Pradhan, 2008). Other plant extracts, such as those derived from milk thistle (Silybum marianum), ginger (Zingiber officinale), and ginseng (Panax ginseng), have also shown promising results in mitigating oxidative stress and improving liver function in experimental studies (Abdel-Moneim *et a*l., 2013; Hsu *et al.,* 2017; Lee *et al*., 2012).

1.2.7 The Kidney

The kidneys are a pair of bean-shaped organs, each approximately the size of a fist. Situated just beneath the rib cage, they are positioned on either side of the spine. The kidneys play a crucial role in various bodily functions. They are responsible for filtering toxins and waste from the blood, such as nitrogen waste (urea), muscle waste (creatinine), and acids. By removing these substances, the kidneys aid in maintaining overall health. Approximately half a cup of blood is filtered by the kidneys every minute.



1.2.7.1 Antioxidant Capacity and the Kidney

The kidneys play a vital role in maintaining homeostasis by filtering waste products from the blood and regulating fluid and electrolyte balance. However, the kidneys are susceptible to oxidative stress, which can lead to cellular damage and impaired renal function (Cachofeiro *et al.,* 2008). Antioxidants have been shown to protect the kidneys from oxidative injury and may have therapeutic potential in the prevention and management of kidney diseases (Rangel-Zúñiga *et al.,* 2022).

1.2.7.2 Oxidative stress and its implications for kidney health

Oxidative stress is a significant contributing factor to the development and progression of various kidney disorders, including chronic kidney disease (CKD), diabetic nephropathy, and acute kidney injury (AKI) (Cachofeiro *et al.,* 2008; Tamadon *et al*., 2022). In the kidneys, excessive free radical production can result from factors such as inflammation, toxin exposure, and metabolic disturbances (Himmelfarb and Hakim, 2003). These free radicals can damage

renal cells, leading to glomerular and tubular injury, impaired filtration and reabsorption, and ultimately, kidney dysfunction (Cachofeiro *et al*., 2008).

The use of animal models, such as Wistar rats, is crucial in preclinical studies aimed at evaluating the safety, efficacy, and mechanisms of action of potential therapeutic agents or natural products (Rang *et al*., 2016). Animal models allow researchers to investigate the effects of interventions on various organs and systems in a controlled experimental setting, while adhering to ethical guidelines and principles of animal welfare.

1.5.6.2 Extraction methods and preparation of *Funtumia elastica* extract

The preparation of plant extracts is an essential step in the study of their biological activities and potential therapeutic applications. While the specific extraction methods used in this study are not provided, researchers commonly employ techniques such as maceration, Soxhlet extraction, or ultrasound-assisted extraction to obtain extracts from plant materials (Sasidharan *et al.,* 2011).

The choice of extraction solvent is also crucial, as it can influence the types of phytochemicals extracted and their bioactivities. Commonly used solvents for plant extractions include water, ethanol, methanol, and other organic solvents, depending on the polarity of the desired compounds (Dai and Mumper, 2010).

It is likely that the researchers in this study used a suitable extraction method and solvent system to obtain the *Funtumia elastica* extract, ensuring the extraction of bioactive compounds responsible for the antioxidant properties.

1.5.7 Antioxidant assays and analytical techniques employed

Evaluating the antioxidant capacity of plant extracts often involves the use of various in vitro and in vivo assays and analytical techniques. Common in vitro antioxidant assays include the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay, and the ferric reducing antioxidant power (FRAP) assay (Gulcin, 2020).

Additionally, researchers may employ analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), or nuclear magnetic resonance (NMR) spectroscopy to identify and quantify the bioactive compounds present in the plant extracts (Sasidharan *et al*., 2011).

1.2.8 Reactive Oxygen Species Generation

Toxic metals have the ability to generate free radicals, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can induce oxidative stress. For example, arsenic has

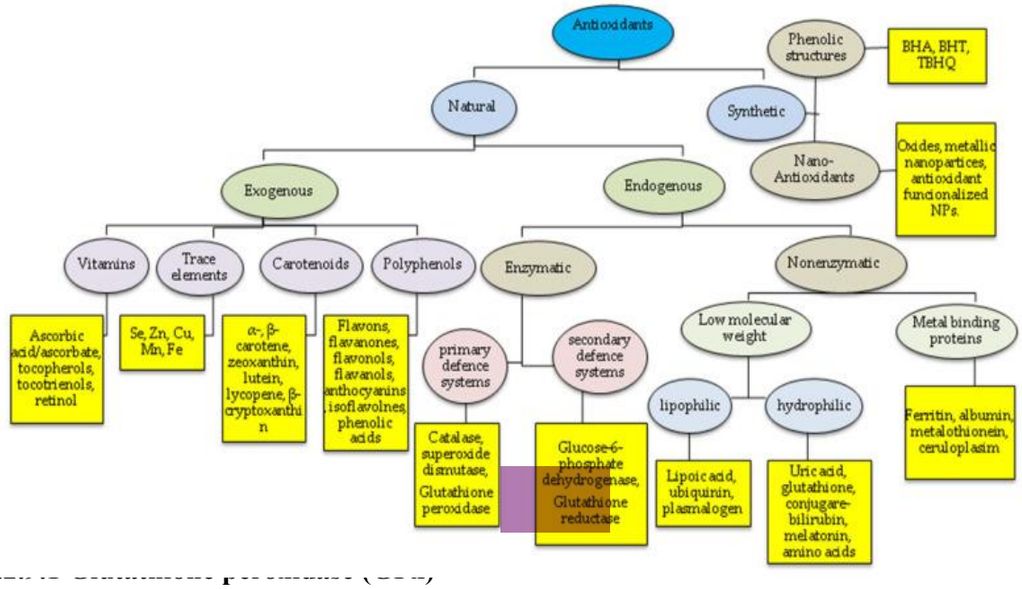
been demonstrated to produce superoxide (O_2^-), oxygen (O_2), nitric oxide (NO), hydrogen peroxide (H_2O_2), and peroxyl (ROO) radicals. Exposure to lead (Pb) significantly decreases antioxidant parameters such as glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), and reduced glutathione (GSH), while increasing oxidative parameters such as malondialdehyde (MDA) and hydrogen peroxide (H2O2) (Wang *et al*., 2013).

ROS and RNS generation induced by hexavalent chromium (Cr(VI)) depletes cellular antioxidant capacity, leading to oxidative stress and subsequent damage to DNA, lipids, and proteins (Yao *et al*., 2008; Aggarwal *et al.*, 2019). Cadmium (Cd) can indirectly generate superoxide (O2−), hydroxyl (OH), and nitric oxide (NO) radicals, overwhelming the cellular antioxidant defense (Imron *et al.*, 2011). Mercury (Hg) is believed to exert its toxic effects on the central nervous system (CNS) and cardiovascular system through increased production of ROS, resulting in reduced activity of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. The high affinity of Hg for thiol (-SH) groups can lead to decreased glutathione peroxidase activity and interfere with intracellular signaling of various receptors. Additionally, methylmercury (Me-Hg) has the ability to activate phospholipase D (PLD), which is implicated in various human cancers and diseases (Fernandes Azevedo *et al*., 2012).

In vivo antioxidant studies often involve measuring oxidative stress markers, such as lipid peroxidation products (e.g., malondialdehyde), antioxidant enzyme activities (e.g., catalase, superoxide dismutase), and levels of non-enzymatic antioxidants (e.g., glutathione) in various tissues or organs of the animal models (Mahmoudi *et al.,* 2022).

1.2.9 Antioxidants

Antioxidants are compounds produced by organisms to counteract oxidative stress caused by an imbalance in reactive oxygen species (ROS). ROS, including hydroxyl radicals generated during redox reactions from aerobic metabolism, can lead to cellular damage (Valko *et al*., 2006). There are various antioxidants, some of which are enzymatic, such as glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase, while others are non-enzymatic, including ascorbic acid and α-tocopherol, among others. In response to environmental stresses, SOD and catalase play a role in defending against ROS-induced damage (Lata and Ahuja, 2003). MDA, a by-product of lipid peroxidation, serves as an indicator of oxidative stress in cells and tissues, and it is associated with cellular injury in both plants and animals (Janero, 1990).



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Glutathione peroxidase (GPx) (EC 1.11.1.9) is a group of enzymes with peroxidase activity that plays a crucial role in protecting organisms from oxidative damage. The primary biochemical function of glutathione peroxidase is to convert lipid hydroperoxides into their respective alcohols, as well as to convert free hydrogen peroxide into water. This enzymatic activity helps safeguard against the harmful effects of oxidative stress.

Glutathione peroxidase (GPx) is an important antioxidant enzyme that plays a crucial role in protecting cells from oxidative stress. Here are some key points about glutathione peroxidase:

Function

GPx catalyzes the reduction of hydrogen peroxide (H2O2) and organic hydroperoxides (ROOH) to water (H2O) and alcohols (ROH), respectively, using glutathione (GSH) as a cofactor.

This reaction helps to neutralize harmful oxidants and prevent oxidative damage to cellular components, such as lipids, proteins, and DNA.

Types of GPx

There are several isoforms of GPx found in different cellular locations and tissues:

GPx1 (cytosolic): Present in most cells and tissues, particularly in erythrocytes.

GPx2 (gastrointestinal): Found in the gastrointestinal tract.

GPx3 (plasma): Secreted into the extracellular space and present in plasma.

GPx4 (phospholipid hydroperoxidase): Plays a role in protecting membranes from oxidative damage.

Role in antioxidant defense

GPx is a key component of the cellular antioxidant defense system, working in conjunction with other enzymes, such as catalase and superoxide dismutase (SOD), to neutralize reactive oxygen species (ROS).

It helps maintain the balance between oxidants and antioxidants, preventing oxidative stress and associated cellular damage.

Importance in health and disease

1. Adequate GPx activity is essential for normal cellular function and overall health.
2. Decreased GPx activity or expression has been associated with various diseases and conditions, including cardiovascular diseases, cancer, neurodegenerative disorders, and aging.
3. GPx levels or activity may be used as a biomarker for oxidative stress and disease risk assessment.

Regulation and factors affecting GPx

GPx activity is influenced by dietary factors, such as selenium (a cofactor for GPx), as well as genetic variations and environmental exposures.

Oxidative stress, inflammation, and certain disease states can also modulate GPx expression and activity.

Glutathione peroxidase plays a crucial role in maintaining cellular redox balance and protecting against oxidative damage. Its activity and expression are tightly regulated and can have significant implications for health and disease prevention.

1.2.9.2 Malondialdehyde (MDA)

MDA is a product of lipid peroxidation and a marker of oxidative stress. It is produced when reactive oxygen species attack polyunsaturated fatty acids in cell membranes.

MDA levels can be measured in various biological samples like plasma, serum, and tissue homogenates using a colorimetric reaction with thiobarbituric acid (TBARS assay). The TBARS assay is simple, rapid, and inexpensive. However, it lacks specificity as substances other than MDA can react with thiobarbituric acid. More specific HPLC methods to measure MDA are available but require sophisticated instrumentation.

Antioxidant compounds and drugs can be assessed in vivo by pretreating animals/humans, inducing oxidative stress, then measuring if MDA levels are attenuated compared to non-treated controls. Lower MDA levels indicate the test compound has antioxidant activity by reducing lipid peroxidation. Strengths of this assay are that it is performed in living systems and provides direct evidence of antioxidant effects against peroxidation. Limitations are lack of specificity of the TBARS assay, and challenges in inducing consistent oxidative stress. Appropriate controls are essential.

MDA is a reasonably good biomarker of in vivo antioxidant activity when applied and interpreted carefully using validated methods.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials

2.1.1 Apparatus

Beakers, conical flask,test tubes,test tube racks, electronic weighing balance,hang gloves, cotton wool, micropipettes(100µl,200µl,1000µl) micropipette tips,foil paper, incubator, mortar and pestle, centrifuge (Techmel and Techmel USA), measuring cylinder (1000ml), microscope, sample container s(plain, EDTA, Heparin) cardboard, syringes (1ml,and 5ml), refrigerator,slides, surgical scissors,forcets,garvage, toilet paper, spectrophotometer (Techmel and Techmel USA), muslin fabric,ice block,filter paper, working bench, electric stove,pot,drug sachets,pH meter

2.1.2 Chemicals

Ethanol, formalin, distilled water, chloroform, EDTA, TCA,TBA,ellamans reagent,pyrogallol, carbonate buffer, phosphate buffer,concentrated hydrochloric acid, bicarbonate, hydrogen peroxide

2.1.3 Reagents

Malondialdehyde(MDA), Glutathione reductase (GSH), Hydrogen peroxidase (H_2O_2), Suphuric acid (6M) H_2SO_4, Carbonate buffer (0.05M), Hydrochloric acid (0.005M), Pyrogallol (20mM), trichloroacetic acid (TCA), Ellman's solution (sodium citrate and 5,5'-dithio-bis-(2-nitrobenzoic acid), and phosphate buffer (sodium dihydrogen phosphate-dihydrate, disodium hydrogen phosphate, and distilled water).

2.2 METHODS

2.2.1 Plant root preparation and extraction

The root sample of *F. elastica* were obtained from Ebhoran in Etsako west local government (LGA), Edo State, Nigeria.

The roots of *Funtuma elastica* was cleaned and air dried at room temperature and then pulverized.

Weighed options of the roots was dissolved in 40% ethanol. The mixture was kept in a shaker at room temperature for 72hrs (Olumese *et al.,* 2018).

The mixture was then filtered and the filtration kept in a rotary evaporator to allow for evaporation of the solvents in order to concentrate the extract.

The dried extract was obtained from the concentrated ethanol extract using a freeze drier

Percentage yield was calculated as;

$$\frac{\text{weight of dried extract obtained}}{\text{weight of plant root used}} \times 100$$

(Ngamkhae *et al*., 2022)

2.2.1 Animal models (Wistar rats) used in the study

In the study investigating the antioxidant capacity of Funtumia elastica extract, the researchers likely employed the use of Wistar rats as an animal model. Wistar rats are an outbred strain of

albino rats commonly used in biomedical research due to their docile nature, ease of handling, and well-characterized physiology (Iannaccone and Jacob, 2009).

2.2.2 Antioxidant Assays

The catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde and glutathione reductase activities were determined in the homogenated kidney and liver tissue samples as described below.

2.2.2.1 Determination of Catalase Activity

Catalase (CAT) activity was estimated by the method described by Cohen *et al.*, (1970).

Preparation of reagents

0.01M KMnO4 was prepared by distilling 0.158g of KMnO4 in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of NaHPO4 and 0.240g of NaH2PO4 was weighed and dissolved in 100ml of distilled water. 6M H2SO4 and 32.3ml of conc. H2SO4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H2O2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H2SO4 and 7ml of 0.01M KMnO4. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The

enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

$$\text{Activity} = \frac{OD/min \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

2.2.2.2 Estimation of Superoxide Dismutase Activity (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

Principle: Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxdiation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Procedure

2.2.2.3 Estimation of Gluthathione Peroxidase (GPx)

This was determined according to Nyman (1959)

Principle: This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Procedure: To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H2O2, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

Activity= OD/min x vt x Df

E x Vs x Y

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

2.2.2.4 Determination of Malondialdehyde (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978)

Principle: Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure: A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

$$\text{MDA (mol/mg protein)} = \frac{A \times V \times 100}{M \times V \times Y}$$

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

2.2.2.5 Estimation of Reduced Glutathione Reductase

The plasma concentration of reduced glutathione (GSH)was determined using the method described by Ellman(1959).

Principle: GSSG in the sample treated with TCA solution and GSH levels were measured using the Ellman method that was modified using 620µl Tris solution at ph 8.2. in this method, thiol residues of GSH oxidize 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) to 5-thio-2-nitrobenzoic acid (TNB) which has an absorbance at 412nm.

Calculation

Concentration of GSH=

$$\frac{A_{test}}{A_{standard}} \times \text{Conc. Of standard}$$

$$\%GSH = (A0 - A1)/A2 \times 100$$

Where A0 = Absorbance of reference sample

A1 = Absorbance of sample

2.2.3 Statistical Analysis

The results are expressed as mean ± SEM, n= 5. The data were subjected to one way analysis of variance (ANOVA) and the significance of differences between mean values was determined using least square difference (LSD). P values less than 0.05 ($P<0.05$) are regarded as statistically significant.

CHAPTER THREE

RESULTS

The chapter presents results showing the antioxidant capacity of the liver and kidney of wistar rats with ethanol extract of *Funtuma elastica.*

Table 3.1: Effect of ethanol extract of *Funtuma elastica* on the antioxidant capacity levels of kidney of wistar rats

GROUPS	MDA(mg/g tissue)	CAT(unit/g tissue)	SOD(unit/g tissue)	GSH(mg/g tissue)	GPX(mg/g tissue)
1A	0.12±0.03	0.35±0.13	0.15±0.016	0.037±0.001	1.68±0.012
2A	0.08±0.0045	0.13±0.05	0.14±0.0125	0.05±0.012	1.62±0.004
3A	0.14±0.02	0.14±0.032	0.14±0.02	0.048±0.012	1.62±0.021
4A	0.10±0.03	0.18±0.02	0.13±0.008	0.042±0.01	1.21±0.016

Data reported as mean ± standard error of mean (SEM),n=3.

3.2 Effect of ethanol extract of *Funtuma elastica* on the antioxidant capacity levels of the liver of wistar rats

GROUPS	MDA(mg/g tissue)	CAT(unit/g tissue)	SOD(unit/g tissue)	GSH(mg/g tissue)	GPX(mg/g tissue)
1A	0.266±0.12	0.27±0.018	0.27±0.039	0.57±0.15	1.64±0.08
2A	0.367±0.15	0.47±0.023[a]	0.27±0.025	0.217±0.16[a]	1.65±0.13
3A	0.111±0.081[a]	0.254±0.014	0.20±0.033	0.207±0.09[a]	1.58±0.21
4A	0.050±0.0015[a]	0.27±0.04	0.23±0.02	0.244±0.102[a]	1.24±0.16

Data reported as mean ± standard error of mean (SEM),n=3.Values with superscript [a] are significantly different ($p \leq 0.05$) from the control group.

The effect of ethanol extract of *Funtuma elastica* on the GSH levels of the liver and kidney of wistar rats are presented in Table 3.2

3.3 RESULT ANALYSIS

From the results shown in the tables above, it is visible that *F. elatica* has no major beneficial or side effects on the antioxidant status of the kidney. We discover that there is no significant alteration ($P>0.05$) in the antioxidant capacity of the kidney when compared to the control group.

Also, in the liver, there is no significant change ($P>0.05$) in the SOD and GPX status. There is a significant decrease ($P<0.05$) in the levels of GSH when compared to the control group. The MDA status of group 3 and group 4 also showed a significant decrease ($P<0.05$) when compared to that of the control group while there was no significant change in the levels of MDA in group

2. There is a significant increase ($P<0.05$) in the CAT status of group 2 when compared to the control group while that of group 3 and 4 remained unchanged.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

From the analysis of the result for antioxidant status of the liver and kidney of wistar rats administered with extracts of *F. elastica,* it is evident that the alteration in the antioxidant status of the kidney and liver of wistar rats did not undergo much alterations.

Ethanol extracts have been shown to impact antioxidant levels and oxidative stress in the kidneys and livers. with ethanol extracts from the plant Funtumia elastica administration significantly decreased antioxidant enzyme levels like GSH, GPx, and SOD in the liver however, caused a significant increase in CAT and MDA levels in the liver (Etengi, 2022). Glutathione, an essential antioxidant, plays a vital role in the liver by neutralizing free radicals and reactive oxygen species (ROS). When the levels of GSH are low, oxidative stress increases as there is not enough antioxidant capacity to counteract the accumulation of ROS. Additionally, GSH is crucial in the liver's detoxification process as it combines with toxins and aids in their elimination from the body. If GSH levels are reduced, this detoxification capacity is impaired, resulting in the buildup of harmful substances in the liver. Consequently, a decrease in GSH levels can make liver cells more vulnerable to damage caused by oxidative stress, environmental toxins, and medications. This heightened susceptibility may elevate the risk of liver diseases such as hepatitis, fatty liver disease, and liver fibrosis.

Low MDA levels are indicative of decreased oxidative stress within the body, resulting in less harm to lipids and cellular structures caused by free radicals and reactive oxygen species. This

implies a lower degree of damage occurring to cell membranes due to lipid peroxidation, ultimately preserving their integrity and functionality. Additionally, reduced MDA levels may help in minimizing inflammation by preventing damage to cells and tissues that could trigger immune responses. Conversely, elevated MDA levels have been linked to various chronic illnesses such as cardiovascular diseases, neurodegenerative disorders, and certain cancers. Therefore, lower MDA levels may suggest a lower risk or severity of these conditions.

4.2 Conclusion

In conclusion, the ethanol extract of Funtuma elastica appears to modulate antioxidant parameters in the liver and kidney of Wistar rats, with varying degrees of impact on different parameters. The observed effects could have implications for the potential use of this extract as an antioxidant intervention or in the management of oxidative stress-related conditions. However, further research is required to fully understand the mechanisms of action and to evaluate the safety and efficacy of this extract in clinical settings.

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APPENDIX

The table below is the result obtained from the statistical data

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SODLIVER	Between Groups	.013	3	.004	1.146	.370
	Within Groups	.044	12	.004		
	Total	.056	15			
SODKIDNEY	Between Groups	.000	3	.000	.164	.919
	Within Groups	.011	12	.001		
	Total	.011	15			
CATLIVER	Between Groups	.127	3	.042	1.368	.299
	Within Groups	.371	12	.031		
	Total	.497	15			
CATKIDNEY	Between Groups	.120	3	.040	3.698	.043
	Within Groups	.130	12	.011		
	Total	.249	15			

GPxLIVER	Between Groups	.458	3	.153	.882	.478
	Within Groups	2.078	12	.173		
	Total	2.536	15			
GPxKIDNEY	Between Groups	.564	3	.188	1.152	.368
	Within Groups	1.957	12	.163		
	Total	2.521	15			
MDALIVER	Between Groups	.250	3	.083	8.736	.002
	Within Groups	.115	12	.010		
	Total	.365	15			
MDAKIDNEY	Between Groups	.008	3	.003	.743	.547
	Within Groups	.042	12	.004		
	Total	.050	15			
GSHLIVER	Between Groups	.359	3	.120	1.659	.228
	Within Groups	.865	12	.072		
	Total	1.225	15			
GSHKIDNEY	Between Groups	.000	3	.000	1.380	.296
	Within Groups	.001	12	.000		
	Total	.002	15			