POSTNATAL ASSESSMENT OF THE ATTENUATION ACTIVITY OF QUERCETIN AND VITAMIN E ON ACCUTANE-INDUCED LIVER AND KIDNEY DAMAGE FOLLOWING PRENATAL EXPOSURE IN WISTAR RATS

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PRESENTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN ANATOMY.

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CERTIFICATION

This is to certify that this research work titled **"POSTNATAL ASSESSMENT OF THE ATTENUATION ACTIVITY OF QUERCETIN AND VITAMIN E ON ACCUTANE-INDUCED LIVER AND KIDNEY DAMAGE FOLLOWING PRENATAL EXPOSURE IN WISTAR RATS"** for the award of a degree of Bachelor of Science (B.Sc.) in Anatomy was carried out by **OMOLUABI GREATNESS AGBONMEDEH** under the supervision of **Dr. Akporobo Ejeguo.** All literatures used in this study have been acknowledged and properly referenced.

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EXTERNAL EXAMINER **DATE**

DEDICATION

To my amazing parents, **Mr. and Mrs. Samson and Stella Omoluabi,** your love, support, encouragement, prayers and sacrifices have shaped me into the person I am today. Every success I achieve is a reflection of your unwavering faith and belief in me, making this journey possible. This work is for you, with all my love and gratitude.

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Finally, to my humble self the real MVP, I say a very big fat thank you, for not giving up despite the ordeals, and for having the resilience and courage to finish strong in spite of everything**.**

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ABBREVIATIONS

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
DNA	Deoxyribonucleic acid
ATP	Adenosine triphosphate
PND30	Postnatal Day 30
SPSS	Statistical Package for the Social Sciences

ABSTRACT

Isotretinoin (Accutane), a retinoid commonly prescribed for severe acne, has been shown to induce oxidative stress and organ toxicity during pregnancy, leading to developmental abnormalities in the fetus. The liver and kidneys are particularly prone to Accutane-induced damage due to their vital roles in detoxification and excretion. Previous studies has shown that quercetin scavenges free radicals and inhibits lipid peroxidation, while vitamin E stabilizes cell membranes and enhances antioxidant defense mechanisms. This study investigated the attenuating effects of quercetin, a natural flavonoid with antioxidant and anti-inflammatory properties, and vitamin E, a lipid-soluble antioxidant, on Accutane-induced liver and kidney toxicity during prenatal development. Pregnant Wistar rats were divided into four groups of five animals each: Control, Accutane only, Accutane + Vitamin E, and Accutane + Quercetin + Vitamin E. Administration was carried out from gestation day 14–21, and the littered fetuses were sacrificed on postnatal day 30 for biochemical and histological analyses. Data was analysed using the SPSS statistical tool. Results showed that exposure to Accutane led to reduced birth weight and crown-rump length compared to other groups, while co-treatment with quercetin and vitamin E improved birth weight comparable to the control. Histological findings of the Accutane-only and Accutane + Vitamin E groups showed periportal inflammation in the liver while the Accutane-only group showed interstitial congestion and tubular swelling in the kidneys, both markedly reduced by co-treatment. In conclusion, the combination of quercetin and vitamin E significantly attenuated Accutane-induced hepatorenal toxicity, highlighting their potential as protective antioxidants for maternal and fetal health.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Prenatal development is a very essential stage in the formation of life. In this stage, cells quickly specialize and differentiates leading to the development of organs. Exposure to any dangerous and harmful substance during this period can lead to long lasting problems for the fetus health and survival (Sadler, 2019). Among the vital organs developed during this process are the liver and kidneys, which are responsible for metabolism, detoxification and waste removal. The fetus liver plays a crucial role for blood cell creation (Hematopoesis) and metabolism, while the kidneys keep fluids and electrolytes in balance. Any disruption during these organs development can lead to a threat on the fetus survival and make the child more likely to get long term illnesses later in life (Moore *et al* ., 2020).

Accutane which is also known as isotretinoin is a synthetic form of vitamin A is a drug used for the treatment of severe and recalcitrant acne conditions (Layton, 2009). While it works well, due to its teratogenic nature, Isotretinoin can lead to birth defects, issues with growth and development and also toxicity in specific organs when taken during pregnancy (Lammer *et al*., 1985). Research shows that isotretinoin mainly causes harm through the generation of reactive oxygen species (ROS), leading to oxidative stress and programmed cell death (Apoptosis) in particular tissues (Küchler *et al*., 2017). The liver and kidneys are more likely prone to damage from isotretinoin because of their primary function of detoxifying and removing waste products from the body (Shirpool *et al*., 2017).

Liver and kidney damage caused by accutane is mainly due to oxidative stress, lipid peroxidation and problems with the mitochondria (Ozkan *et al*., 2013). During fetal development, there is a higher chance of oxidative harm when the natural oxidant defenses are

not fully formed (Halliwell & Gutteridge, 2015). This has led to the numerous research of protective, defensive substances that can counteract and work against the harmful,damaging effects of isotretinoin. Due to the protective qualities of Quercetin and Vitamin E, researchers have shown increasing interest in these naturally occuring antioxidants. Quercetin, a flavonoid which is mostly found in fruits and vegetables is well known for its anti-inflammatory and antioxidant properties (Boots *et al*., 2008). Vitamin E, a lipid-soluble antioxidant gives stability to cell membranes and disrupts lipid peroxidation chain reactions (Brigelius-Flohé & Traber, 1999). Though the protective abilities against oxidative damage of both antioxidants have been individually shown, their combined synergistic effects against prenatal hepatorenal toxicity caused by Accutane have limited research. Therefore, this study seeks to examine and evaluate the attenuating effects of Quercetin and Vitamin E individually and in combination against Accutane-induced liver and kidney toxicity during prenatal development.

1.2 Aim of Study

To evaluate the protective effects of Quercetin and Vitamin E against postnatal Accutane-induced liver and kidney toxicity following prenatal exposure

1.3 Objectives of Study

The following are the objectives of this study:

1. To assess the effects of Accutane on the liver and kidneys of developing fetuses.
2. To determine the protective potential of Vitamin E on Accutane-induced organ damage.
3. To evaluate the combined effects of Quercetin and Vitamin E in reducing oxidative stress and toxicity.
4. To compare the histological changes among treatment groups.
5. To establish whether the combination therapy offers better protection than Vitamin E alone.

1.4 Significance of the Study

1. Provides insight into the toxic effects of Accutane on the liver and kidneys during prenatal development.
2. Highlights the importance of antioxidants in protecting fetal organs from oxidative damage.
3. Demonstrates the potential therapeutic role of Quercetin and Vitamin E in reducing drug-induced toxicity.
4. Aims to proffer basics for more research into the beneficial effects of combination of quercetin and vitamin E against accutane toxicity in the development of the liver and kidney.

1.5 Statement of Research Problem

1. Accutane (Isotretinoin) is effective for severe acne but can cause serious side effects.
2. It is a known teratogen, leading to fetal malformations during pregnancy.
3. Prolonged exposure might result in hepatic and renal damage in the fetus.
4. Antioxidants like Quercetin and Vitamin E have shown potential to reduce oxidative injury and counteract the effect of several toxicants.
5. However, limited studies have explored their combined protective effect against Accutane-induced liver and kidney toxicity during prenatal development.

(Mitchell *et al*., 2019; El-Hefnawy *et al*., 2021; Brigelius-Flohé & Traber, 2019; Li *et al*., 2020).

1.6 Justification of the Study

Birth defects caused by Accutane due to its teratogenicity is a major health issue around the world, especially from the large number of Isotretinoin intake among women who are able to have children. The liver and kidneys, organs that are vital for the processes of metabolic

activities and removal of toxins (Detoxification) are especially at risk to the harmful effects of isotretinoin. Damage to these organs while a baby is developing in the womb could negatively affect the growth and survival of the baby while also making them more likely to experience health problems later in life (Moore *et al.*, 2020). Although isotretinoin teratogenic caused birth defects have been shown in multiple research, there is little information about how it affects the liver and kidney specifically before birth. Moreover, though Quercetin and Vitamin E are known antioxidants, not much research has been done on how well they work together to prevent liver and kidney damage caused by drugs.

This study therefore will justify as a way to offer new understanding of how natural antioxidants might protect against Accutane-induced toxicity during fetal development. The results may also help in creating more secure treatment approaches and preventive measures for exposed groups.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Prenatal development is the complex, biological process which shows the various stages of growth throughout the fertilization of the egg evolving into a fully formed fetus. The development process consists of two main phases, beginning with the embryonic stage which is from week 1 - week 8 and at this stage, organ formation (organogenesis) takes place and then continues to the second phase known as the fetal stage from week 9 - week 40, at this stage the organ systems undergo growth, differentiation and functional development (Moore *et al*., 2020). During these stages of organ formation and development, cell division, specialization and tissue reshaping (morphogensis) occur creating the foundational and basic structure for all vital organs.

The liver and kidneys are some of the first and vital organs to form during prenatal development developing during the first trimester. The liver originates from the hepatic diverticulum during week 3 to 4 of pregnancy and handles blood cell production (hematopoesis), metabolic functions, glycogen storage and detoxification or waste elimination (Sadler, 2019). Since the liver is a very vital organ in the body even from prenatal development, any disruption in its development can cause metabolic dysfunction and impaired detoxification systems that can lead to birth defects affecting organ structure and functionality. The kidneys originate from the metanephric mesenchyme and ureteric bud, forming functional working nephrons between week 12 and 14 (Moore *et al*., 2020). Kidneys in the fetus regulates fluid and electrolyte levels, produces amniotic fluid and are essential for the removal of metabolic waste products. Problems during this process of kidney development can lead to defective nephron creation which reduces the body's ability to handle stress increasing the chances of having high blood pressure and kidney disease later in life.

The liver and kidneys show high sensitivity to oxidative stress during fetal development due to their high metabolic activity and fetal antioxidant defenses not fully formed. Over production of reactive oxygen species (ROS) may cause damage to lipids, proteins and nucleic acids,

leading to cell death (apoptosis), organ failure and problems in organ development (Halliwell & Gutteridge, 2015).

Antioxidants, produced from both within the body (endogenous) and obtained from outside the body (exogenous) help protect fetal organs by preserving their structural integrity. Quercetin, the flavonoid existing in high amounts in fruits and vegetables shows strong and potent antioxidant properties with high anti-inflammatory and anti-apoptotic qualities. It removes reactive oxygen species (ROS) while limiting lipid peroxidation and boosting the activity of natural endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Boots *et al*., 2008; D'Andrea, 2015). Vitamin E, an antioxidant that dissolves in fat making it a lipid-soluble antioxidant, stabilizes cell membranes while stopping lipid peroxidation from spreading which protects the liver and kidney cells from damage caused by oxidation (Brigelius-Flohé & Traber, 1999; Niki, 2014). When these antioxidants are taken during pregnancy as a form of supplement, they pass through the placenta aiding the development of the fetus's liver and kidneys while protecting against damage from oxidative stress.

Accutane(Isotretinoin), a strong teratogenic medication known to cause birth defects mainly cause harm to fetal development through its effect on oxidative stress mechanisms (Küchler *et al*., 2017). The liver and kidneys, which are vital for the processing and removal of drugs are directly exposed to the toxic metabolic byproduct. The antioxidant properties of Quercetin and Vitamin E protect against Accutane-induced liver and kidney damage by reducing oxidative stress, maintaining cell integrity and supporting organ strength during important stages of growth.

2.2. Drug of Study: Accutane (Isotretinoin)

Fig 2.1: Accutane (Isotretinoin) (Dermatology Clinic of Laredo).

Accutane, also known by its generic name Isotretinoin, is a synthetic derivative of vitamin A (retinol) belonging to the class of compounds known as retinoids. It was first developed in the 1970s and officially introduced for medical use in 1982 by Hoffmann-La Roche under the brand name Accutane (Leyden *et al*., 1983). Initially, isotretinoin was explored for the treatment of skin keratinization disorders such as ichthyosis and keratosis follicularis, before its potent effects on severe acne were discovered (Shalita, 1988). Accutane gained interest as a highly effective oral medication for severe recalcitrant acne, a form of acne resistant to normal therapy. It acts by reducing sebaceous gland size and sebum production, inhibiting keratinization, and decreasing the presence of propionibacterium acnes, all of which contribute to its therapeutic success (Layton, 2009; Zouboulis *et al*., 2014).

Isotretinoin, known chemically as 13-cis-retinoic acid, is closely related to all-trans-retinoic acid, or tretinoin. These compounds work their magic by binding to nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which in turn regulate gene expression tied

to cell growth, differentiation, and apoptosis (Kang *et al*., 2010). However, while Accutane has its clinical advantages, it also comes with some serious side effects, such as teratogenicity, liver dysfunction, and kidney toxicity, especially when taken in high doses or for extended periods (Lammer *et al*., 1985; El-Hefnawy *et al*., 2021). The way the drug is metabolized in the liver and its ability to induce oxidative stress make these organs particularly vulnerable to toxicity (Li *et al*., 2020).

2.2.2 Morphology and Production of Accutane

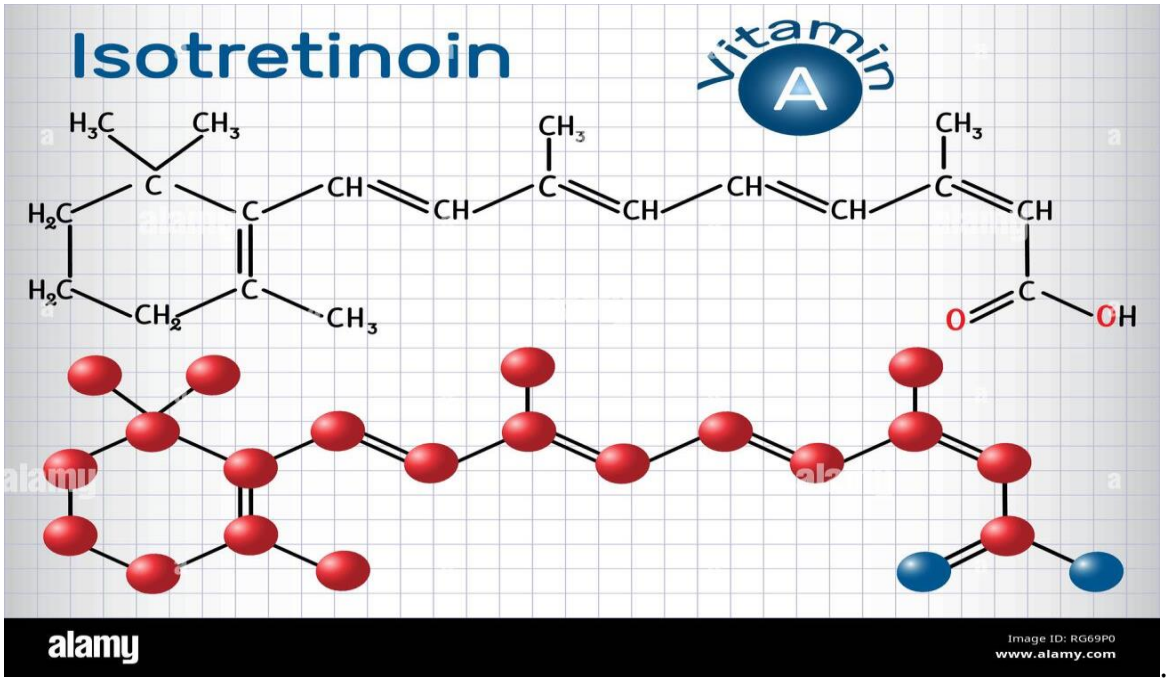


Fig 2.2: Chemical structure of Accutane (Isotretinoin)(PubChem (NCBI), 2014).

Isotretinoin, also known as 13-cis-retinoic acid, is a yellow to orange crystalline compound that's both lipid-soluble and sensitive to light. Its molecular formula is $C_{20}H_{28}O_2$ and it has a molecular weight of 300.44 g/mol (Goodman & Gilman, 2018). In terms of structure, it is an isomer of all-trans-retinoic acid (tretinoin), which is the active form of vitamin A. The unique

cis-configuration around the 13th carbon atom is what gives isotretinoin its special pharmacological properties (Kang *et al*., 2010).

Typically, isotretinoin is synthesized through a chemical process that involves isomerizing retinoic acid derivatives or vitamin A (retinol) sourced from either natural or synthetic origins. In industrial settings, this process starts with the oxidation of β-ionone, a compound derived from carotenoids, followed by a series of reactions that create the necessary 13-cis configuration for isotretinoin (Roche, 1982). Because isotretinoin is sensitive to light and prone to oxidation, it needs to be formulated and stored in low-light, airtight conditions to keep it stable. It's usually produced in capsule form, often made of soft gelatin, and comes in concentrations of 10 mg, 20 mg, or 40 mg, dissolved in neutral oils like soybean oil or beeswax (FDA, 2020).

The drug is mainly metabolized in the liver, where it undergoes oxidation and isomerization to produce metabolites such as 4-oxo-isotretinoin, which also have biological activity (Lammer *et al*., 1985). These metabolites are eventually excreted through the kidneys, which is why the liver and kidneys are particularly susceptible to oxidative damage caused by isotretinoin.

2.2.3 Phytochemicals and Other Components of Accutane

Unlike natural compounds derived from plants, Accutane (Isotretinoin) is a synthetic derivative of Vitamin A (retinoic acid) and not a phytochemical based drug. Its active component, 13-cis-retinoic acid, belongs to the retinoid family compounds structurally related to retinol (Vitamin A) that regulate cell growth, differentiation, and apoptosis (Kane *et al*., 2008). Chemically, isotretinoin's molecular formula is $C_{20}H_{28}O_2$ and it functions by binding to retinoic acid receptors (RARs) and retinoid X receptors (RXRs) in the nucleus. These receptors are essential for controlling the expression of genes that manage cell growth and differentiation, especially in epithelial tissues and sebaceous glands (Layton, 2009).

Once isotretinoin is processed in the liver, it transforms into several active metabolites, such as 4-oxo-isotretinoin, all-trans-retinoic acid, and 4-oxo-retinoic acid. These metabolites are responsible for both the beneficial and harmful effects of the drug (Zouboulis, 2001). They can build up in the liver and kidneys, leading to oxidative stress by producing reactive oxygen species (ROS) and reducing levels of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Shin *et al*., 2020). Due to this oxidative potential, isotretinoin can impact lipid metabolism, the integrity of cell membranes, and enzyme activity in the liver and kidneys. So, while it's effective for treating acne, the chemical makeup of isotretinoin is a significant factor in its potential to cause birth defects and liver or kidney toxicity, particularly when taken during pregnancy.

2.2 4 Therapeutic Benefits of Accutane

Accutane (Isotretinoin) remains one of the most effective and clinically established treatments for severe, recalcitrant nodulocystic acne that does not respond to conventional therapies like antibiotics or topical retinoids (Layton, 2009). It works by reducing sebaceous gland size and sebum production, which directly limits the environment that supports acne-causing bacteria such as Cutibacterium acnes (Zouboulis, 2001). Beyond its anti-acne effect, it also helps normalize keratinization by encouraging proper differentiation of epithelial cells and reducing follicular plugging, which is a major player in the formation of acne lesions (Goldsmith *et al*., 2012). Plus, it has anti-inflammatory properties that help calm down inflammatory cytokines and lower neutrophil activity at the sites of acne (Kang *et al*., 2008).

On the clinical front, isotretinoin has been investigated for treating other skin and cancer-related conditions, including rosacea, psoriasis, and certain types of skin cancers, thanks to its ability to manage cell growth and differentiation (Nassif *et al*., 2010).

However, despite all these benefits, the use of isotretinoin comes with significant risks, particularly its severe teratogenic effects and potential for systemic toxicity, which can include liver and kidney damage. The drug's active metabolites can lead to oxidative stress and lipid peroxidation, causing structural and functional changes in the liver and kidneys (El-Hefnawy *et al*., 2021). As a result, the advantages of this treatment must be carefully balanced against the necessity for strict medical oversight and, in the case of pregnancy, a complete ban due to its risks to fetal development.

2.3 Antioxidants

2.3.1 Antioxidant: Vitamin E

Fig 2.3: Natural Sources of Vitamin E (NIH Office of Dietary supplements, 2024).

Vitamin E refers to a group of eight naturally occurring fat-soluble compounds, which include four tocopherols (α, β, γ, δ) and four tocotrienols. Among these, α-tocopherol stands out as the most biologically active and is the predominant form found in human tissues (Traber & Atkinson, 2007). It serves as one of the body's key antioxidants, playing a vital role in safeguarding cell membranes and lipid-rich organelles from oxidative damage. As a chain-

breaking antioxidant, Vitamin E halts the spread of lipid peroxidation by donating a hydrogen atom to lipid radicals, which stabilizes them and prevents further oxidative stress. It's primarily stored and functions within cell membranes, especially in the mitochondria, endoplasmic reticulum, and plasma membrane, where it protects polyunsaturated fatty acids (PUFAs) from peroxidation.

In addition to its antioxidant properties, Vitamin E also affects gene expression, immune regulation, and enzymatic activity, making it crucial for maintaining cellular integrity and metabolic balance. It works in harmony with other antioxidants like vitamin C and selenium, which help to regenerate oxidized vitamin E back to its active form, ensuring ongoing protection against oxidative damage (Brigelius-Flohé & Traber, 1999).

2.3.1.1 Morphology and Production of Vitamin E

Vitamin E refers to a group of fat-soluble compounds consisting mainly of tocopherols and tocotrienols, with α-tocopherol being the most biologically active and predominant form in human tissues (Traber & Stevens, 2011). Morphologically, Vitamin E is a light yellow, oily liquid that is insoluble in water but soluble in fats and organic solvents. It is heat-stable but sensitive to light and oxygen, which can degrade its potency over time (Brigelius-Flohé & Traber, 1999). Production of Vitamin E occurs through both natural and synthetic processes. Naturally, it is obtained from plant oils, nuts, seeds, and green leafy vegetables, where it is synthesized by plants through the shikimate and mevalonate pathways. These biochemical pathways produce phytyl side chains and chromanol rings, which combine to form tocopherols and tocotrienols (Mène-Saffrané & DellaPenna, 2010).

Industrial production of Vitamin E often involves extraction from vegetable oils such as soybean, sunflower, and wheat germ oils, followed by purification using chromatographic techniques. Alternatively, synthetic Vitamin E, known as dl-α-tocopherol, is manufactured

chemically from trimethylhydroquinone and isophytol through condensation and hydrogenation reactions (Schneider, 2005). The natural (d-α-tocopherol) form, however, is considered more potent and bioavailable than the synthetic racemic mixture (Huang *et al*., 2019). Because of its lipid-soluble nature, Vitamin E is efficiently incorporated into cell membranes and lipoproteins, where it performs its protective antioxidant functions, especially within organs like the liver and kidneys that are prone to oxidative damage.

2.3.1.2 Phytochemicals and Other Components of Vitamin E

Vitamin E is a family of compound of eight structurally related molecules divided into two main groups:

Tocopherols (α-, β-, γ-, and δ-)

Tocotrienols (α-, β-, γ-, and δ-)

Each of these molecules features a chromanol ring, which plays a crucial role by donating hydrogen to neutralize free radicals, along with a hydrophobic phytyl tail that helps anchor the molecule into cell membranes (Brigelius-Flohé & Traber, 1999). Among them, α-tocopherol stands out as the most biologically active and is the most prevalent form found in human plasma, making it the primary form for supplementation and research (Traber, 2007).

The phytochemical strength of Vitamin E lies in its antioxidant-rich structure, especially the hydroxyl group on the chromanol ring. This group actively scavenges reactive oxygen species (ROS) and helps prevent lipid peroxidation in biological membranes (Niki & Traber, 2012). This structural feature enables Vitamin E to stabilize free radicals by donating a hydrogen atom, converting them into less reactive species.

Besides tocopherols and tocotrienols, some Vitamin E formulations also include cofactors and carriers like ascorbyl palmitate (a vitamin C ester) or selenium compounds. These additions

work together to boost its antioxidant capabilities and regenerative effects (Burton & Traber, 1990). Although tocotrienols are less common in our diets, their unique unsaturated side chains allow them to penetrate tissues more effectively, providing neuroprotective, hepatoprotective, and renoprotective benefits (Sen *et al.*, 2006). Together, these components play a vital role in maintaining the stability of cellular membranes, reducing oxidative stress, and enhancing our immune and enzymatic defenses. These processes are essential for protecting important organs like the liver and kidneys from oxidative damage caused by toxins or medications such as Isotretinoin (Accutane).

2.3.1.3 Therapeutic and Health Benefits of Vitamin E

Vitamin E stands out as one of the body's most potent lipid-soluble antioxidants, playing a crucial role in keeping our cells intact and shielding our tissues from oxidative damage. Its main therapeutic advantage is its ability to prevent lipid peroxidation which is the harmful process where free radicals cause attack on cell membranes, leading to inflammation, cell death, and organ dysfunction (Traber & Atkinson, 2007). In the liver, Vitamin E acts as a source of protection for liver cells against damage from drug-induced oxidative stress, such as that caused by isotretinoin or acetaminophen. It achieves this by neutralizing reactive oxygen species (ROS), stabilizing cell membranes, and maintaining the activity of enzymes essential for detoxification (Abdel-Moneim *et al.*, 2016). Research has shown that Vitamin E supplementation can help reduce liver fibrosis, inflammation, and degeneration by lowering levels of ALT and AST enzymes key indicators of liver injury. When it comes to the kidneys, Vitamin E helps counteract oxidative damage caused by nephrotoxic substances, enhancing glomerular function and reducing tubular degeneration (Al-Dosari, 2011). Its antioxidant properties are further supported by its anti-inflammatory and immunomodulatory effects, which help preserve normal kidney structure and function. Studies indicate that Vitamin E can

lower urea and creatinine levels, thereby improving kidney performance in models exposed to toxins or suffering from disease (Boonsanit *et al*., 2006).

But beyond the liver and kidneys, the health benefits of Vitamin E is also linked to heart protection, brain health, and reproductive advantages, due to its ability to regulate gene expression, prevent platelet clumping, and boosting of nitric oxide availability (Brigelius-Flohé & Traber, 1999).

2.3.2 Antioxidant: Quercetin

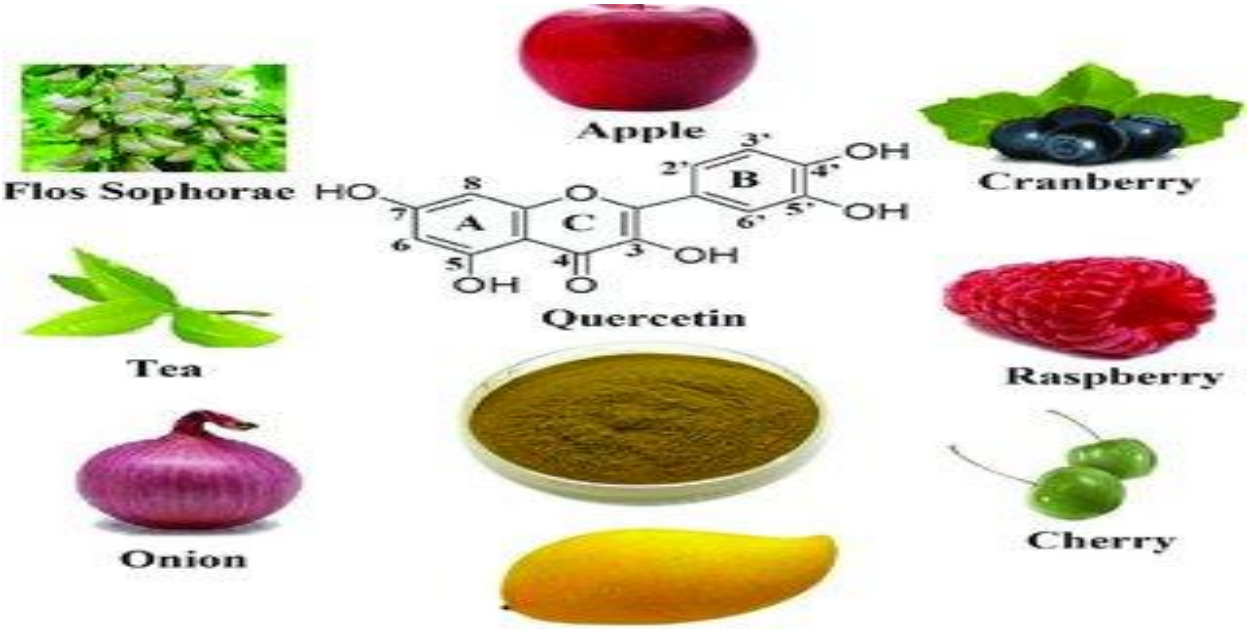


Fig 2.4: Chemical structure and natural sources of quercetin (NIH Office of Dietary supplements, 2024).

Quercetin is a naturally occurring flavonoid that can be found in a variety of fruits, vegetables, leaves, and grains especially in onions, apples, berries, citrus fruits, and green tea. It's one of the most abundant dietary antioxidants, falling under the flavonol subclass of polyphenolic compounds, known for their powerful ability to scavenge free radicals and reduce inflammation (Boots *et al*., 2008). Structurally, quercetin possesses five hydroxyl groups on its flavone backbone, which contributes to its strong antioxidant potential. It is often present in plants as

glycosides, meaning it's attached to sugar molecules like glucose or rutinose, enhancing its solubility and bioavailability in biological systems (Panche *et al*., 2016).

The biological significance of quercetin lies in its ability to manage oxidative stress and inflammation, two key players in cellular and organ damage. It works by neutralizing reactive oxygen and nitrogen species, binding to transition metals, and boosting our own antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Li *et al*., 2016). Additionally, quercetin has anti-apoptotic, anti-fibrotic, and vasoprotective properties, making it a effective substance for preventing liver and kidney toxicity, especially from drugs like isotretinoin (Accutane). Its ability for crossing cell membranes and affecting intracellular signaling pathways gives it a unique advantage in keeping tissues healthy and repairing them during oxidative stress.

2.3.2.1 Morphology and Production of Quercetin

Quercetin, also known as 3,3′,4′,5,7-pentahydroxyflavone, is a vibrant yellow crystalline compound that falls under the flavonol category of flavonoids. Structurally, it features two aromatic rings (A and B) linked by a three-carbon bridge that forms a heterocyclic C ring. This unique arrangement gives quercetin its remarkable chemical properties and impressive antioxidant abilities (Panche *et al*., 2016). The numerous hydroxyl (-OH) groups on its rings boost its capacity to donate hydrogen atoms, which is crucial for its role in scavenging free radicals.

In its pure state, quercetin presents itself as a bitter yellow powder that doesn't dissolve in cold water but mix well with alcohol and lipids. It rarely appears in its free (aglycone) form in nature. Instead, it's mostly found as glycosides, where one or more sugar molecules like glucose, galactose, or rutinose are attached to it (Ross & Kasum, 2002). These glycosidic forms are more stable and enhance its solubility and absorption in the body. Plants synthesize quercetin

through the phenylpropanoid pathway, starting from the amino acid phenylalanine. This pathway involves several enzymatic steps that transform phenylalanine into p-coumaroyl-CoA, which then interacts with malonyl-CoA via the chalcone synthase enzyme to create naringenin chalcone, a crucial intermediate. Additional enzymatic modifications eventually lead to the production of quercetin (Ferreyra *et al*., 2012).

Commercially, quercetin is extracted from plant sources that are rich in flavonoids, such as the flower buds of Sophora japonica (Japanese pagoda tree), Ginkgo biloba leaves, onion skins, and tea leaves. The extraction process typically involves solvents like ethanol, methanol, or aqueous-alcohol solutions, followed by purification using chromatographic techniques (Wiczkowski *et al*., 2008).

2.3.2.2 Phytochemicals and Other Components of Quercetin

Quercetin is one of the most extensively researched bioflavonoids, celebrated for its strong antioxidant, anti-inflammatory, and cell-protective qualities. It falls under the category of polyphenolic compounds and is often found alongside other phytochemicals that boost its biological effects (Boots *et al*., 2008). In the plants, quercetin typically combine with other flavonoids like kaempferol, myricetin, rutin, and catechins. Together, they work in uniformly to scavenge reactive oxygen species (ROS) and lower oxidative stress. These compounds share similar structures with quercetin and collectively enhance the antioxidant defense system in both plants and living tissues (Panche *et al*., 2016).

Phytochemically, quercetin contains five hydroxyl groups that give it high reactivity toward free radicals, enabling it to donate hydrogen atoms and stabilize reactive molecules. This structure is key to its ability to neutralize superoxide anions, hydroxyl radicals, and peroxynitrite species (Formica & Regelson, 1995). In addition to its antioxidant role, quercetin exhibits metal chelating properties, binding to transition metals like iron and copper that

catalyze free radical generation through Fenton reactions. This ability helps prevent lipid peroxidation and protein oxidation, protecting cellular membranes and organ integrity (Li *et al*., 2020).

Furthermore, quercetin interacts with other natural antioxidants such as vitamin C, vitamin E, and glutathione (GSH), regenerating them and boosting their activity. This synergistic effect enhances redox balance and improves protection against drug-induced toxicity, particularly in hepatic and renal tissues exposed to harmful compounds like isotretinoin (Accutane) (El-Hefnawy *et al*., 2021).

2.3.2.3 Therapeutic and Health Benefits of Quercetin

Quercetin has gained a reputation for its impressive range of therapeutic benefits, thanks to its powerful antioxidant, anti-inflammatory, antiviral, and liver-protecting properties. By neutralizing free radicals and preventing lipid peroxidation, it plays a vital role in safeguarding our essential organs, like the liver and kidneys (Li *et al*., 2020). One of the major roles of quercetin is its ability to shield against organ damage caused by medications. Research shows that quercetin can significantly lower oxidative stress in the liver and kidneys, help maintain enzyme balance, and protect cellular health in animals exposed to harmful substances like isotretinoin (Accutane) (El-Hefnawy *et al*., 2021). It does this by boosting the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while also reducing malondialdehyde (MDA) levels, which is a key indicator of lipid peroxidation (Boots *et al*., 2008).

Beyond its antioxidant effects, quercetin also has anti-inflammatory capabilities. It works by lowering the levels of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, which helps reduce tissue inflammation in the liver and kidneys, areas often affected by Accutane toxicity

(Panche *et al*., 2016). Additionally, quercetin disrupts NF-κB signaling pathways, which helps minimize cellular damage and encourages tissue healing (Formica & Regelson, 1995).

Moreover, quercetin plays a role in cellular protection and regeneration, boosting mitochondrial function and keeping cell membranes stable. It aids in liver detoxification by enhancing bile flow and preventing fat buildup in liver cells (Li *et al*., 2020). In the kidneys, it helps maintain glomerular filtration, reduces tubular damage, and limits the infiltration of inflammatory cells, ensuring that the kidneys perform better even under toxic stress (El-Hefnawy *et al*., 2021). It's important to note that when quercetin is paired with vitamin E, they work together to create powerful antioxidant effects. Vitamin E helps protect lipid membranes, while quercetin not only regenerates but also boosts the antioxidant power of vitamin E. This synergistic ability provides a stronger defense against oxidative stress caused by Accutane, which is crucial for protecting both the mother and the developing fetus during pregnancy (Mitchell *et al*., 2019). In a broader sense, quercetin's health benefits go beyond general antioxidation, it also serve as a versatile bio-protectant, helping to restore biochemical balance, reduce inflammation, and enhance the structure of liver and kidney tissues that have suffered from oxidative damage.

2.3.3 Combined Health Benefits of Antioxidants (Vitamin E and Quercetin)

The combined use of Vitamin E and Quercetin has gained increasing attention in experimental and clinical studies due to their synergistic antioxidant and cytoprotective actions. Both compounds possess distinct yet complementary mechanisms in combating oxidative stress and maintaining cellular integrity. Vitamin E, being a lipid-soluble antioxidant, primarily protects biological membranes and lipoproteins from peroxidation by scavenging lipid radicals and interrupting the propagation of oxidative chain reactions. Quercetin, a potent flavonoid operates mainly within aqueous environments of the cytoplasm where it neutralizes reactive oxygen species (ROS), enhances endogenous antioxidant enzyme activities, and modulates inflammatory signaling pathways (Boots *et al*., 2008; Li *et al*., 2016). When co-administered, Vitamin E and Quercetin demonstrate enhanced free radical scavenging potential compared to their individual effects. Quercetin can regenerate oxidized forms of Vitamin E (tocopheroxyl radicals) back into their active antioxidant state, thereby prolonging Vitamin E's protective function in cell membranes (Zhang *et al*., 2011). This interaction amplifies overall cellular defense by maintaining a balanced redox state and reducing lipid peroxidation and protein oxidation. Additionally, the combination exerts anti-inflammatory, hepatoprotective, and renoprotective effects by modulating key molecular pathways such as NF-κB and Nrf2, which regulate oxidative stress response and inflammatory gene expression (Szymanska *et al*., 2018; Wang *et al*., 2020).

2.3.4 Combined Effects of Accutane and Antioxidants (Vitamin E and Quercetin)

Accutane (Isotretinoin) is a potent retinoid known for its therapeutic efficacy in treating severe acne and other dermatological conditions. However, its use has been linked to dose-dependent hepatic and renal toxicities, primarily mediated through oxidative stress, lipid peroxidation, and inflammatory responses (Alotaibi *et al*., 2020; Khan *et al*., 2021). The metabolism of Accutane in the liver leads to the generation of reactive oxygen species (ROS), which can

disrupt cellular homeostasis, damage membrane lipids, and alter antioxidant enzyme balance. Similarly, in the kidneys, Accutane may impair filtration function and elevate oxidative markers, leading to tissue injury and altered biochemical parameters (Omar *et al*., 2016).

Co-administration of antioxidants such as Vitamin E and Quercetin has shown promising protective effects against Accutane-induced hepatic and renal toxicity. These antioxidants mitigate oxidative stress by neutralizing ROS, enhancing the activities of endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and restoring normal redox balance in hepatic and renal tissues (Hosseinzadeh *et al*., 2017; Sharma *et al*., 2020). Vitamin E, due to its lipid-soluble nature, effectively stabilizes cell membranes and prevents lipid peroxidation initiated by Accutane metabolism, thereby protecting hepatocytes and renal tubular cells. Quercetin complements this effect by scavenging aqueous phase radicals, modulating inflammatory cytokine production, and improving mitochondrial function (Li *et al*., 2016). Their combination enhances overall protection, not only preventing oxidative damage but also promoting tissue regeneration and functional recovery.

Experimental studies have demonstrated that Vitamin E and Quercetin co-treatment significantly reduces elevated liver enzymes (ALT, AST, ALP) and kidney markers (urea, creatinine) in models of retinoid-induced toxicity (Eken *et al*., 2019; Wang *et al*., 2020).

2.4 Organ(s) of Study: The Liver

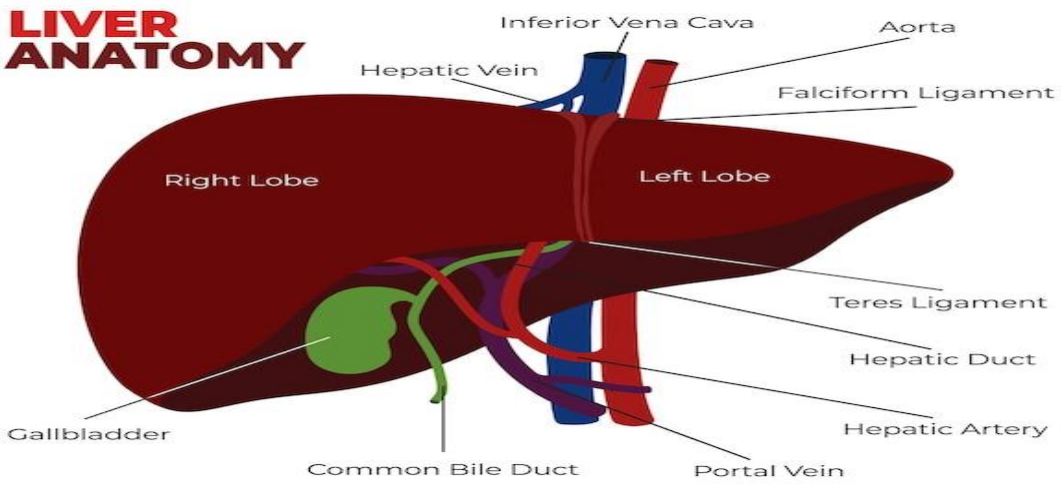


Fig 2.5: Well-labelled diagram of the Liver

2.4.1 Gross Anatomy of the Liver

The liver is the largest internal organ and gland in the human body, weighing approximately 1.4–1.6 kg in adults and located in the right upper quadrant of the abdominal cavity, just below the diaphragm (Guyton & Hall, 2021). It is wedge-shaped, reddish-brown in color, and enclosed by a thin connective tissue capsule known as Glisson's capsule, which provides structural support and protection. Anatomically, the liver is divided into two main lobes; the larger right lobe and the smaller left lobe which is separated by the falciform ligament, which attaches the liver to the anterior abdominal wall. On its visceral (inferior) surface, two smaller lobes, the caudate and quadrate lobes, are also identifiable (Tortora & Derrickson, 2020).

The liver receives a dual blood supply which means approximately 75% of its blood comes from the portal vein, which delivers nutrient-rich but oxygen-poor blood from the gastrointestinal tract, while about 25% is supplied by the hepatic artery, carrying oxygenated blood from the systemic circulation (Ross & Pawlina, 2019). Blood from both sources mixes in the hepatic sinusoids and eventually drains into the central veins, which merge to form the hepatic veins that empty into the inferior vena cava. On the inferior surface lies the porta hepatis,

the liver's "gateway," where the hepatic artery, portal vein, and bile duct enter and exit, collectively known as the portal triad.

2.4.2 Embryology of the Liver

The liver is one of the first organs to form and function during embryonic development. It originates from the endoderm of the foregut region around the third week of gestation (Sadler, 2021). The initial liver development begins with the formation of the hepatic diverticulum, also known as the liver bud, which grows out from the ventral wall of the foregut into the surrounding septum transversum mesenchyme, a mass of mesodermal tissue that later contributes to the diaphragm and ventral mesentery.

The cranial portion of the hepatic diverticulum gives rise to the liver parenchyma (hepatocytes and bile ducts), while the caudal portion forms the gallbladder and cystic duct (Moore et al., 2020). As the hepatic cords (primitive hepatocytes) proliferate and invade the septum transversum, they form an intimate association with the vitelline and umbilical veins, which later develop into the hepatic sinusoids, establishing the organ's early vascular network. By the sixth week, the liver becomes a major hematopoietic organ, producing red and white blood cells during fetal life. This hematopoietic activity peaks between the sixth and twelfth weeks of development and then declines as the bone marrow assumes this role. During this stage, the liver occupies a large portion of the abdominal cavity, accounting for nearly 10% of the total fetal weight (Sadler, 2021). As development continues, hepatocytes begin synthesizing bile by the twelfth week of gestation, which is then secreted into the duodenum through the developing biliary tract. The interaction between endodermal and mesenchymal cells is crucial in regulating the differentiation of hepatocytes and the formation of the bile ducts.

2.4.3 Histological Architecture of the Liver

Histologically, the liver is composed of thousands of lobules, which are its functional units. Each lobule is roughly hexagonal in shape and consists of plates or cords of hepatocytes (liver cells) radiating outward from a central vein (Ross & Pawlina, 2019). These hepatocytes are polygonal cells with abundant cytoplasm and a centrally located nucleus, specialized for metabolism, detoxification, and secretion of bile.

At each corner of the lobule lies the portal triad, which includes three key structures:

1. A branch of the hepatic artery,

2. A branch of the portal vein and

3. A bile ductule (Junquiera & Carneiro, 2020).

Between the hepatocyte plates are hepatic sinusoids which features are wide, thin-walled vascular channels lined by fenestrated endothelial cells and Kupffer cells (specialized macrophages). These sinusoids facilitate the exchange of substances between the blood and liver cells. The Kupffer cells remove debris, old red blood cells, and pathogens, maintaining the liver's immune and detoxification roles. Blood from the hepatic artery and portal vein flows through the sinusoids toward the central vein, while bile produced by hepatocytes flows in the opposite direction — toward the bile duct of the portal triad. The bile canaliculi, small grooves between adjacent hepatocytes, serve as channels for bile collection before it drains into the bile ducts. Additionally, the space of Disse, located between the hepatocytes and sinusoidal endothelium, allows plasma exchange and is home to Ito cells (stellate cells), which store vitamin A and play a role in fibrosis during liver injury.

2.4.4 Functions of the Liver

The liver performs a wide range of vital metabolic, synthetic, and detoxification functions that are essential for maintaining homeostasis. It serves as a central hub for processing nutrients, neutralizing toxins, and regulating biochemical balance across multiple organ systems (Guyton & Hall, 2021).

1. Metabolism of Carbohydrates, Proteins, and Lipids:

The liver regulates blood glucose by storing glucose as glycogen (glycogenesis) and releasing it during fasting (glycogenolysis or gluconeogenesis). It also plays a key role in amino acid metabolism, including deamination of amino acids and urea formation for excretion of ammonia. In lipid metabolism, the liver synthesizes cholesterol, triglycerides, and lipoproteins, which are essential for energy and cell membrane formation.

2. Detoxification and Biotransformation:

One of the liver's most critical roles is detoxification. It converts lipid-soluble toxins, drugs, and metabolic by-products into water-soluble forms for excretion through bile or urine. This process, carried out mainly by the cytochrome P450 enzyme system, is crucial in protecting the body from harmful compounds such as Accutane (isotretinoin) and other retinoids (Nelson & Cox, 2021).

3. Synthesis of Plasma Proteins and Enzymes:

The liver synthesizes vital plasma proteins such as albumin, which maintains oncotic pressure, and clotting factors (e.g., fibrinogen, prothrombin). It also produces enzymes like ALT (alanine aminotransferase) and AST (aspartate aminotransferase), which serve as clinical biomarkers of hepatic function and injury.

4. Bile Production and Secretion:

The liver produces bile, a fluid essential for the emulsification and absorption of dietary fats in the small intestine. Bile also serves as a primary route for excreting waste products, including bilirubin and cholesterol.

5. Storage of Vitamins and Minerals:

The liver stores fat-soluble vitamins (A, D, E, K) and essential minerals like iron and copper, releasing them as needed to maintain metabolic balance.

6. Immune Function and Blood Filtration:

The Kupffer cells, specialized macrophages in the liver sinusoids, remove pathogens, old red blood cells, and debris from the bloodstream, contributing to immune defense and detoxification.

2.4.5 Hepatotoxicity and Mechanisms of Liver Toxicity

Hepatotoxicity refers to any form of liver injury or functional impairment caused by exposure to drugs, chemicals, or other toxic agents. Since the liver is the body's main detoxification organ, it is especially vulnerable to toxic insults, particularly from lipophilic drugs like Isotretinoin (Accutane) that undergo extensive metabolism within hepatocytes (Jaeschke *et al*., 2021).

1. Mechanisms of Drug-Induced Liver Injury (DILI):

Drug-induced hepatotoxicity can occur through two main mechanisms:

- Direct (Intrinsic) Toxicity: predictable, dose-dependent damage (e.g., acetaminophen toxicity).
- Idiosyncratic Toxicity: unpredictable and not dose-dependent, often linked to genetic or immune factors.

Accutane primarily exhibits dose-dependent intrinsic toxicity, driven by oxidative stress and lipid peroxidation in hepatocytes.

2. Role of Oxidative Stress:

Accutane metabolism in the liver generates reactive oxygen species (ROS), which attack cell membranes, mitochondrial DNA, and proteins. Excessive ROS overwhelms the liver's antioxidant defense systems (e.g., SOD, CAT, GSH), leading to lipid peroxidation, cell membrane damage, and hepatocyte necrosis (Yasmeen *et al*., 2020).

3. Inflammation and Immune Response:

Damaged hepatocytes release damage-associated molecular patterns (DAMPs) that activate Kupffer cells, triggering the release of inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This inflammatory cascade amplifies tissue injury and fibrosis if exposure continues.

4. Mitochondrial Dysfunction:

Accutane interferes with mitochondrial respiration and ATP production, resulting in energy depletion and activation of apoptotic pathways via cytochrome c release (Wang *et al*., 2018). This contributes to hepatocyte apoptosis and loss of functional tissue.

5. Enzyme Leakage and Biomarkers of Injury:

The disruption of hepatocyte membranes causes leakage of ALT, AST, and ALP into circulation which are key indicators of hepatocellular damage. Elevated levels of these enzymes are consistent with hepatocellular necrosis and inflammation.

6. Histopathological Changes:

Histological findings in Accutane-induced liver injury often include periportal inflammation, ballooning degeneration, sinusoidal congestion, and necrosis. Chronic exposure may progress to fibrosis or steatosis due to impaired lipid metabolism.

2.5 Organ of study(s): The Kidney

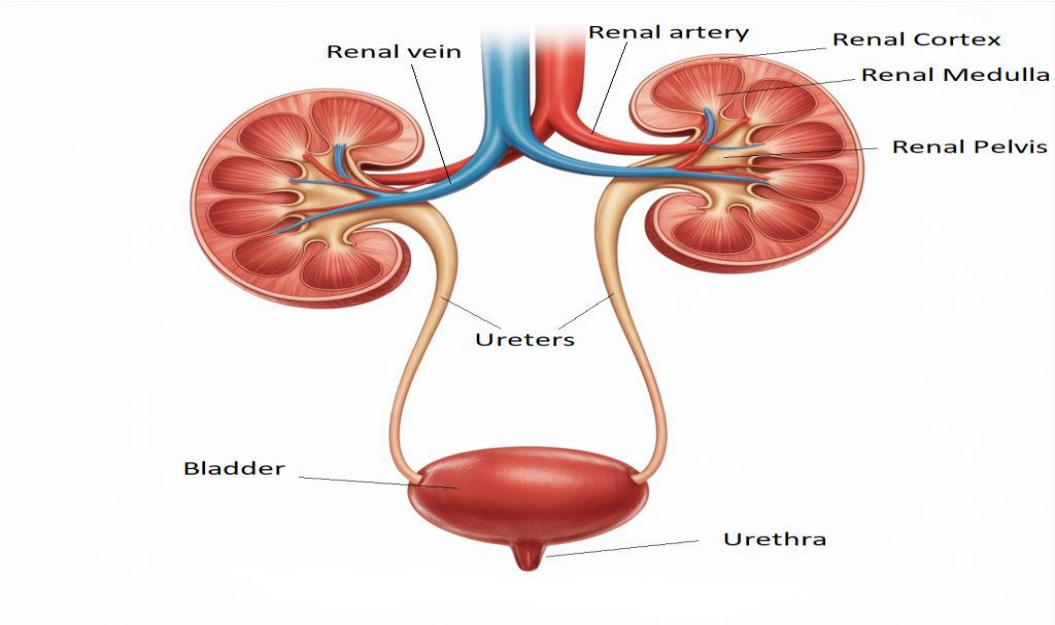


Fig 2.6: Well-labelled diagram of the kidneys

2.5.1 Gross Anatomy of the Kidney

The kidneys are two bean-shaped organs located retroperitoneally on either side of the vertebral column, extending roughly from the level of the T12 to L3 vertebrae. The right kidney is usually positioned slightly lower than the left due to the presence of the liver.

Each kidney measures about 11–13 cm in length, 5–7 cm in width, and 2.5 cm in thickness, with an average weight of 120–170 g in adults. Each kidney has two surfaces (anterior and posterior), two borders (lateral and medial), and two poles (upper and lower). The lateral border is convex, while the medial border is concave and contains the renal hilum, where structures such as the renal artery, renal vein, and ureter enter or leave the organ. Internally, the kidney is divided into two major regions:

- The renal cortex: a lighter outer region containing renal corpuscles and convoluted tubules.

- The renal medulla: a darker inner region composed of renal pyramids, each with a renal papilla projecting into a minor calyx. Several minor calyces unite to form a major calyx, which drains into the renal pelvis, the funnel-shaped upper part of the ureter.

Each kidney receives blood from the renal artery, a direct branch of the abdominal aorta, and drains into the renal vein, which empties into the inferior vena cava. The kidneys are enclosed by a fibrous capsule, surrounded by perirenal fat, and further enclosed by the renal fascia, which anchors them to surrounding structures.

2.5.2 Embryology of the Kidney

The development of the kidney, or nephrogenesis, is a complex and well-coordinated process that occurs through three successive stages: pronephros, mesonephros, and metanephros. Each stage represents a more advanced and functional structure, with only the final one persisting as the permanent kidney.

1. Pronephros (Early, Nonfunctional Stage):

The earliest and most primitive form of the kidney. Appears around the 4th week of embryonic development in the cervical region. Consists of a few cell clusters and tubules that soon degenerate and disappear. Although nonfunctional in humans, it serves as a foundation for the development of the next stage.

2. Mesonephros (Interim Functional Kidney):

Develops caudal to the pronephros and functions temporarily from the 4th to 8th week. Formed by mesonephric tubules and a mesonephric (Wolffian) duct, which drain into the cloaca. The mesonephric tubules form a simple filtration system, connecting to glomerular capillaries derived from the dorsal aorta. In males, part of the mesonephros persists and contributes to the formation of reproductive structures such as the epididymis, vas deferens, and efferent ducts.

3. Metanephros (Permanent Kidney):

Begins development around the 5th week and becomes functional by the 10th week. Arising from two key embryonic structures, the ureteric bud which is an outgrowth of the mesonephric duct, which forms the ureter, renal pelvis, calyces, and collecting ducts and the metanephric mesenchyme (blastema), derived from intermediate mesoderm, which gives rise to the nephrons (glomeruli, Bowman's capsule, tubules, and loops of Henle). Reciprocal inductive interactions between these two structures are essential for proper kidney formation and failure leads to congenital anomalies such as renal agenesis.

4. Postnatal Maturation:

At birth, the kidney is lobulated, and nephrogenesis is complete; however, functional maturation continues after birth. The lobulations disappear as the cortex thickens and new nephrons are no longer formed.

2.5.3 Histological Architecture of the Kidney

The kidney's microscopic structure is highly organized to perform its filtration and regulatory functions efficiently. It is primarily made up of millions of functional units called nephrons, supported by an intricate network of blood vessels and connective tissue.

Each kidney has two main regions under the microscope:

1. Renal Cortex:

The outer region containing renal corpuscles and convoluted tubules. The renal corpuscle is composed of a Bowman's capsule enclosing a network of capillaries called the glomerulus. Blood enters through the afferent arteriole and leaves via the efferent arteriole. The proximal convoluted tubule (PCT), rich in mitochondria and microvilli, reabsorbs most of the filtered water, ions, and nutrients. The distal convoluted tubule (DCT) has fewer microvilli and is involved in selective secretion and reabsorption under hormonal control.

2. Renal Medulla:

Composed mainly of loops of Henle and collecting ducts arranged in parallel bundles, forming the renal pyramids. The loop of Henle has descending and ascending limbs crucial for the kidney's ability to concentrate urine. Collecting ducts gather filtrate from multiple nephrons and pass through the medulla to open at the renal papillae into the minor calyces.

3. Interstitial Tissue and Blood Supply:

The interstitial tissue supports the nephron structure and contains fibroblasts, immune cells, and interstitial capillaries. Blood supply follows a sequence through the renal artery to the segmental arteries then through the interlobar arteries to the arcuate arteries then through the interlobular arteries to the afferent arterioles and then to the glomeruli. After filtration, blood exits via the efferent arterioles, forming the peritubular capillaries and vasa recta, which play key roles in reabsorption and countercurrent exchange.

4. Juxtaglomerular Apparatus (JGA):

Located near the vascular pole of the glomerulus. Composed of macula densa cells, juxtaglomerular (JG) cells, and extraglomerular mesangial cells. It regulates blood pressure and glomerular filtration rate through renin secretion.

2.5.4 Functions of the Kidney

The kidneys are essential organs that perform multiple vital functions necessary for maintaining homeostasis. Their functions can be broadly categorized as follows:

1. Excretory Function:

The kidneys remove metabolic waste products from the bloodstream, including urea, creatinine, uric acid, and other nitrogenous wastes. This excretion helps maintain the body's nitrogen balance and prevents the accumulation of toxic substances.

2. Regulation of Water and Electrolytes:

The kidneys regulate the volume and composition of body fluids by controlling the levels of sodium, potassium, chloride, calcium, and phosphate. This function ensures proper fluid balance and electrolyte homeostasis.

3. Acid-Base Balance:

By excreting hydrogen ions (H^+) and reabsorbing bicarbonate ions (HCO_3^-), the kidneys help maintain the blood pH within the normal physiological range, ensuring metabolic stability.

4. Blood Pressure Regulation:

The kidneys participate in blood pressure regulation through the renin-angiotensin-aldosterone system (RAAS). They modulate blood volume and vascular resistance, which are crucial for maintaining adequate systemic blood pressure.

5. Erythropoiesis Regulation:

The kidneys produce erythropoietin, a hormone that stimulates the bone marrow to increase red blood cell production, ensuring sufficient oxygen-carrying capacity of the blood.

6. Detoxification:

The kidneys assist in detoxifying certain drugs and metabolites, contributing to the body's defense against harmful substances.

7. Vitamin D Activation:

The kidneys convert inactive vitamin D (cholecalciferol) into its active form, calcitriol, which is essential for calcium and phosphate homeostasis, and thus for proper bone health.

8. Gluconeogenesis:

During periods of fasting, the kidneys can produce glucose from non-carbohydrate precursors, supplementing the liver in maintaining blood glucose levels.

2.5.5 Renal Toxicity and Mechanisms of Kidney Toxicity

Renal toxicity, also known as nephrotoxicity, refers to the damage to the kidneys caused by exposure to toxic substances, drugs, or environmental chemicals. The kidneys are particularly vulnerable because they receive a high volume of blood and are involved in concentrating and excreting substances, which can lead to accumulation of toxins.

Common Causes of Renal Toxicity

1. Drugs:

- Aminoglycoside antibiotics (e.g., gentamicin)
- NSAIDs (non-steroidal anti-inflammatory drugs)
- Chemotherapeutic agents (e.g., cisplatin, ifosfamide)

2. Environmental and Industrial Chemicals:

- Heavy metals such as lead, mercury, and cadmium
- Organic solvents and pesticides

3. Endogenous Toxins:

- High levels of uric acid (hyperuricemia)
- Hemoglobin (from hemolysis) or myoglobin (from rhabdomyolysis)

Mechanisms of Kidney Toxicity:

The mechanisms by which toxic substances damage the kidneys include:

1. Direct Tubular Toxicity:

Certain drugs or chemicals directly damage renal tubular epithelial cells, causing cell death and loss of function. Example: Aminoglycosides bind to proximal tubular cells, disrupting lysosomal function and leading to necrosis.

2. Oxidative Stress:

Toxins induce overproduction of reactive oxygen species (ROS) in renal cells, causing lipid, protein, and DNA damage. This leads to apoptosis or necrosis of kidney cells.

3. Inflammation and Immune-Mediated Injury:

Some substances trigger an immune response or inflammatory cytokine release in the kidney, resulting in tissue injury. Example: Drug-induced interstitial nephritis.

4. Hemodynamic Changes:

Drugs like NSAIDs reduce renal blood flow by inhibiting prostaglandin synthesis, leading to ischemia and hypoxic injury, especially in the renal medulla.

5. Crystal-Induced Nephropathy:

Certain drugs (e.g., acyclovir, methotrexate) can form crystals that precipitate in renal tubules, causing obstruction and damage.

6. Mitochondrial Dysfunction:

Some toxins impair mitochondrial function in tubular cells, reducing ATP production and compromising active transport, leading to cell injury.

2.6 Patterns of Liver Injury Due to Accutane (Isotretinoin)

Accutane (Isotretinoin) induces distinct patterns of hepatic injury, primarily associated with its metabolism and the generation of reactive oxygen species (ROS) in hepatocytes. These patterns reflect the structural and functional disturbances within the liver during toxicity and are often identified through biochemical changes and histopathological findings (Abd-El-Ghany *et al*., 2020; Li *et al*., 2021).

1. Hepatocellular (Parenchymal) Injury:

This is the most common pattern of Accutane-induced liver damage. It occurs when hepatocytes the main functional cells of the liver undergo degeneration or necrosis due to oxidative stress and lipid peroxidation.

- Biochemical indicators: Elevated serum ALT and AST levels.
- Histological features: Ballooning degeneration of hepatocytes, periportal inflammation, and focal necrosis.
- Mechanism: ROS generated from Accutane metabolism damage hepatocyte membranes, disrupt mitochondrial function, and impair protein synthesis.

2. Cholestatic Injury:

Accutane may also cause cholestasis, a condition where bile flow is impaired within the liver.

- Biochemical indicators: Increased ALP and bilirubin levels.
- Histological features: Accumulation of bile pigments within hepatocytes and bile canaliculi, with mild portal inflammation.

- Mechanism: Retinoid-induced disruption of bile acid transport and canalicular integrity, leading to impaired bile excretion.

3. Mixed Hepatocellular Cholestatic Injury:

Some cases exhibit overlapping features of both hepatocellular and cholestatic injury.

- Biochemical indicators: Elevated ALT, AST, ALP, and bilirubin concurrently.
- Histological features: Areas of hepatocyte necrosis with concurrent cholestasis and portal inflammation.

4. Steatotic (Fatty) Change:

Long-term exposure to Accutane may cause hepatic steatosis due to altered lipid metabolism.

- Histological features: Accumulation of lipid droplets within hepatocytes (micro- or macrovesicular steatosis).
- Mechanism: Retinoid interference with peroxisome proliferator-activated receptor (PPAR) signaling, leading to fat accumulation in the liver.

5. Inflammatory and Fibrotic Changes:

Prolonged inflammation activates Kupffer cells and stellate cells, promoting fibrosis.

- Histological features: Deposition of collagen fibers in periportal and perisinusoidal regions.
- Mechanism: Chronic oxidative stress and cytokine release stimulate fibrogenesis, potentially progressing toward cirrhosis if unchecked.

2.7 Pattern of Kidney Injury Due to Accutane (Isotretinoin)

Accutane (isotretinoin), is primarily metabolized by the liver. However, it can have renal effects, although nephrotoxicity is rare. The pattern of kidney injury typically includes:

1. Functional Alterations:

Mild changes in renal function may occur, including slight elevations in serum creatinine and blood urea nitrogen (BUN). These are usually reversible upon discontinuation of the drug.

2. Tubular Effects:

Some studies suggest disturbances in tubular reabsorption, potentially leading to electrolyte imbalances (e.g., mild hypercalcemia) and altered urine concentrating ability.

3. Immune-Mediated Injury (Rare):

Accutane can occasionally trigger drug-induced interstitial nephritis, an immune-mediated reaction characterized by Inflammatory infiltration of the renal interstitium and mild proteinuria and hematuria.

4. Mechanisms of Renal Injury:

- Direct tubular toxicity: Retinoids may affect tubular epithelial cells at high concentrations.
- Immune modulation: Accutane can trigger hypersensitivity reactions, leading to inflammation in the kidney.
- Altered lipid metabolism: Hypertriglyceridemia induced by isotretinoin can contribute indirectly to renal stress.

2.8 Oxidative Stress in Liver and Kidney Toxicity

Oxidative stress refers to a state where there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, leading to cellular damage. Both

the liver and kidneys are highly susceptible to oxidative stress because of their roles in detoxification, metabolism, and excretion of xenobiotics.

Mechanism of Oxidative Stress

1. Generation of Reactive Oxygen Species (ROS):

Drugs, environmental toxins, and metabolic processes can increase ROS, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH).

2. Cellular Damage:

ROS react with lipids, proteins, and DNA, causing lipid peroxidation, protein oxidation, and DNA strand breaks. This leads to membrane instability, enzyme inactivation, and cell death.

3. Organ Vulnerability:

- Kidneys: High blood flow and active tubular transport concentrate toxins, making tubular cells particularly sensitive to ROS.
- Liver: As the main detoxifying organ, hepatocytes metabolize xenobiotics, generating ROS as byproducts that can overwhelm antioxidant defenses.

4. Consequences of Oxidative Stress:

- Cellular apoptosis or necrosis
- Functional impairment of liver and kidneys
- Progression of nephrotoxicity or hepatotoxicity
- Altered metabolic and excretory functions

Relevance to Drug-Induced Toxicity:

Drugs like Accutane (isotretinoin) can induce oxidative stress indirectly by altering lipid metabolism, generating free radicals, and reducing antioxidant capacity.

2.9 Mechanisms of Accutane-Induced Oxidative Damage

Accutane (isotretinoin), can induce oxidative stress in the liver and kidneys, contributing to cellular and tissue injury. The mechanisms involved include:

1. Enhanced Reactive Oxygen Species (ROS) Production

Accutane metabolism generates reactive oxygen species, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals. The accumulation of ROS overwhelms endogenous antioxidant defenses, leading to oxidative damage to cellular components.

2. Lipid Peroxidation

ROS attack polyunsaturated fatty acids in cellular membranes, initiating lipid peroxidation. This disrupts membrane integrity, affecting hepatocytes and renal tubular epithelial cells, impairing their function.

3. Protein Oxidation

ROS can oxidize amino acid residues in proteins, leading to enzyme inactivation and structural damage. This affects key metabolic and transport enzymes in the liver and kidney, further impairing organ function.

4. DNA Damage

Excessive ROS can induce single- and double-strand DNA breaks, potentially triggering apoptosis in affected cells.

5. Impairment of Antioxidant Defenses

Accutane may reduce endogenous antioxidant levels (e.g., glutathione, superoxide dismutase, catalase), weakening the cell's ability to neutralize ROS. This creates a vicious cycle where oxidative damage perpetuates itself.

6. Indirect Effects via Lipid Metabolism Alteration

Accutane can increase serum triglycerides and cholesterol, which may promote oxidative stress in vascular, hepatic, and renal tissues..

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials:

Ceramic plates, syringe, weighing scale, chloroform, surgical gloves, cotton wool, scissors, beakers, universal bottles, plain bottles, plastic cages, sawdust, formalin, slides, paraffin wax, normal saline, oral gavage, pipette and microscope, refrigerator.

Chemicals:

Quercetin, Vitamin E, Accutane

All reagents and chemicals were of analytical grade.

Experimental Animals:

Twenty (20) pregnant Wistar rats weighing averagely 155g were used in this study. Animals were kept in standard cages in the animal house at the Department of Anatomy, University of Benin, Benin city, Edo State and were acclimatized for 2 weeks. They were fed daily with Grower's mash (manufactured by Premier feed Mills Co LTD, a subsidiary of Flour Mills of Nigeria Plc) and water *ad libitum*. The rats were weighed weekly throughout the duration of the experiment using a digital weighing scale calibrated in gram and recorded to the nearest whole number. They were then mated with male rats during appropriate stages of the estrous cycle. Vaginal smears were carried out to determine pregnancy followed by a three-week gestation period after a confirmation of pregnancy. All animal procedures was performed in accordance with the approved protocols and adhering to the guidelines for the ethical treatment and use of laboratory animals in research (Chikerie *et al*., 2015).

3.2 Experimental Design

Twenty adult pregnant Wistar rats were assigned into four groups based on their weight A-D comprising of five rats per group. All the rats had free access to feed and water. Administration lasted for seven days starting from gestation day 14 for each pregnant rat.

Table 3.1: Experimental Design

GROUPS	TREATMENT
GROUP A	Served as the control group
GROUP B	10mg/kg body weight of Accutane
GROUP C	10mg/kg body weight of Accutane + 50mg/kg body weight of quercetin + 500mg/kg body weight of vitamin E
GROUP D	10mg/kg body weight of Accutane + 500 mg/kg body weight of vitamin E

All administration was done via oral route using an oral gavage.

3.3 Monitoring of Estrous Cycle and Mating

The estrous cycle in rats is a recurrent reproductive cycle lasting approximately 4–5 days, consisting of four distinct phases: proestrus, estrus, metestrus, and diestrus. Each phase is characterized by specific cellular and hormonal changes that influence reproductive physiology and tissue responses:

- Proestrus: The period preceding ovulation, characterized by proliferation of the uterine lining and high estrogen levels.

- Estrus: The phase of sexual receptivity and ovulation; estrogen peaks, and the female is fertile.
- Metestrus: The transitional phase following estrus, where progesterone levels begin to rise and estrogen levels decline.
- Diestrus: The quiescent phase, marked by low estrogen and progesterone, preparing the reproductive tract for the next cycle.

Daily vaginal smears were performed to monitor cytological changes and determine the phase of the cycle. Proper timing of experiments according to the estrous phase is essential because hormonal fluctuations during different phases can affect oxidative stress, drug metabolism, and tissue susceptibility to injury. By ensuring all females were in the same phase at the start of treatment, variability in experimental outcomes was minimized.

Mating

Mating was conducted to study reproductive-stage-related responses and to standardize gestational age for treatment administration. Female rats confirmed to be in estrus, the phase of peak fertility and were paired with male rats in a separate mating cage during the dark phase when rats are most active. The presence of a vaginal plug, observed the following morning indicated successful copulation and was designated as day 0 of gestation. Females were then returned to their individual cages and monitored daily. Mating during the estrus phase is critical because it ensures synchronized pregnancy timing, which allows consistent evaluation of drug effects at defined stages of gestation or reproductive cycle. This detailed monitoring of the estrous cycle and controlled mating procedure ensured uniform reproductive status across experimental animals, reducing variability and enhancing the reliability of biochemical and histological analyses in liver and kidney tissues.

3.4 Sourcing and Collection of Drugs

Accutane(Isotretinoin), Quercetin and Vitamin E (alpha-tocopherol) were obtained from a registered pharmacy in Awka, Anambra state, Nigeria and transported to Benin city, Edo state.

3.5 Drug Preparation and Method of Administration

The drugs and antioxidants were dissolved in water at a standardized drug-to-solvent ratio calculated according to their respective milligram concentrations. Administration of drug was done using an oral gavage to ensure precise treatment delivery with specific dosage of 10mg/kg of Accutane, 50mg/kg of quercetin and 500mg/kg of vitamin E.

3.6 Sacrifice of Animals and Sample Collection

The rats were weighed at the beginning, during (on a weekly basis) and at the end of the study using a weighing balance. After the treatment period, rats were allowed to litter and the offspring was kept until PND30 before sacrifice under chloroform anesthesia. They were sacrificed by cervical dislocation, the organs harvested and blotted free of blood and weighed using digital weighing scale calibrated in grams. The harvested liver and kidney organs were fixed in 10% formal saline and tissues processed for light microscopic examination.

3.7 Histological and Statistical Analysis

3.7.1 Histological Analysis

Liver and kidney tissues were processed following standard histopathological protocols. Samples were fixed in 10% formal saline, dehydrated, cleared, and embedded in paraffin wax. Sections of 5 μm thickness were cut using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E) for general tissue morphology. Stained sections were examined under a light microscope to assess cellular architecture, tissue integrity, and the presence of any pathological alterations induced by treatment.

3.7.1.1 Photomicrography

A research microscope with attached digital camera was used to examine the sections. Photomicrograph of the tissue sections were taken at different magnifications.

3.7.2 Statistical Analysis

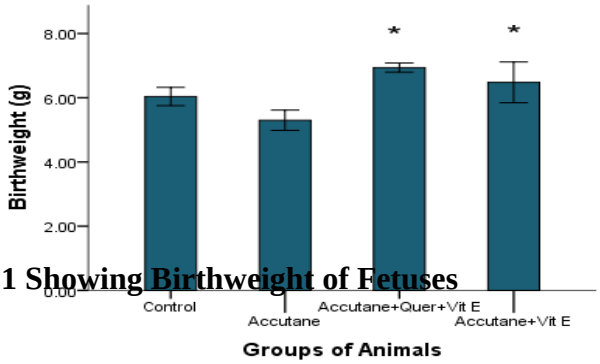
All quantitative data were analyzed using SPSS version 22. One-way analysis of variance (ANOVA) was used to determine differences among experimental groups, followed by Tukey's post hoc multiple comparison test to identify specific group differences. Data were expressed and a p-value < 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Birth Weight

Results obtained showed that there was significant difference (P>0.05) in the birth weight of the fetuses across experimental groups.



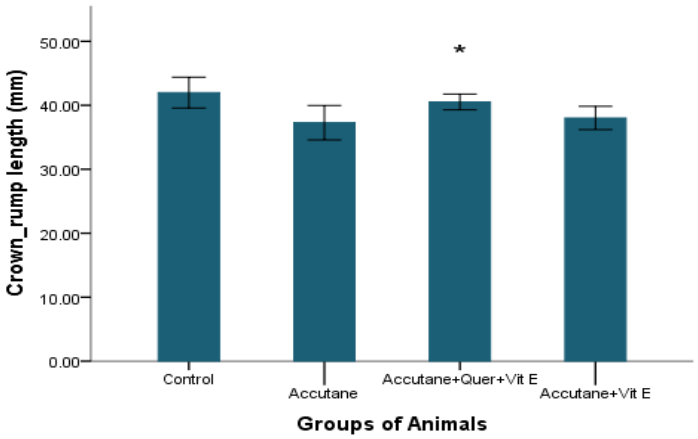
Bar Chart 4.1 Showing Birthweight of Fetuses

@ indicates p<0.05 compared to control

*indicates p<0.05 compared to Accutane only

4.2 Crown- rump length

Results obtained showed that there was significant difference (P>0.05) in the birth weight of the fetuses across experimental groups.

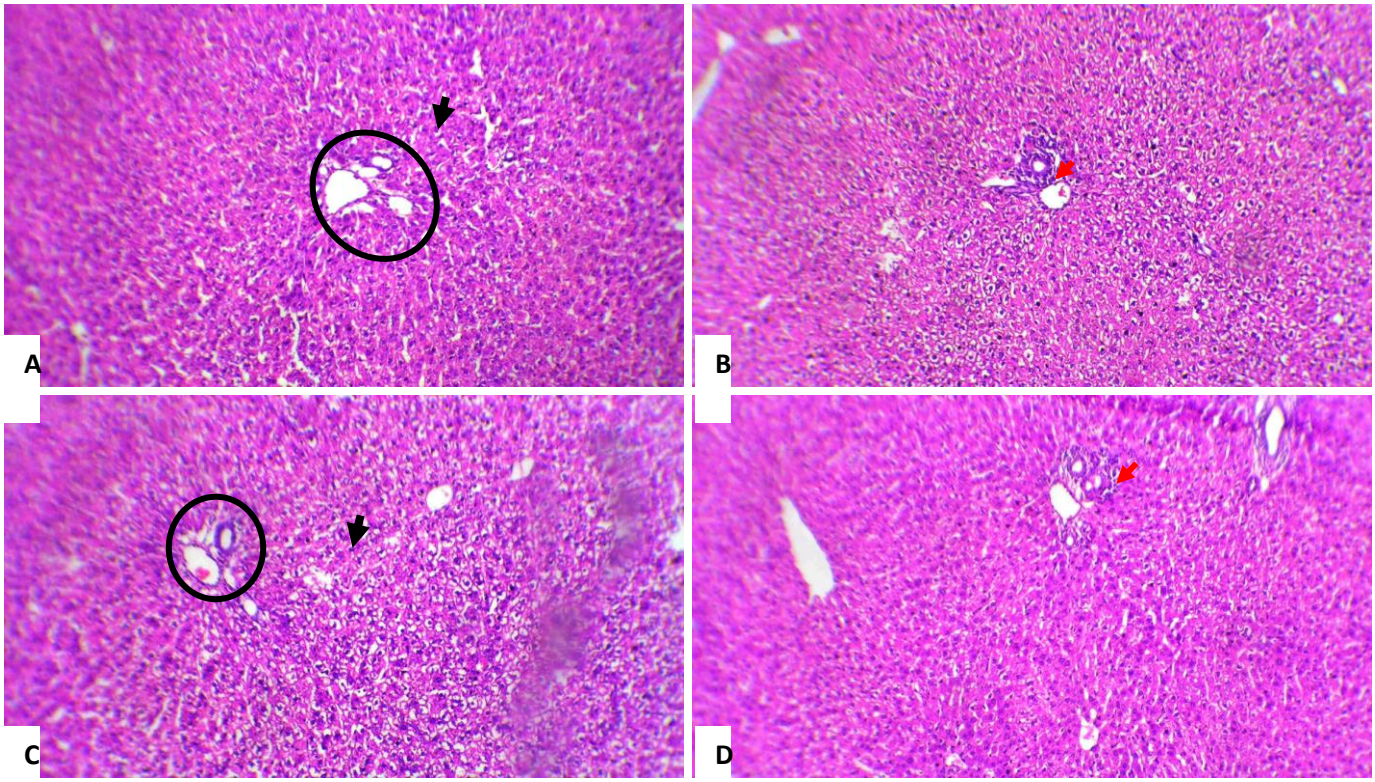


Bar Char

@ indicates p<0.05 compared to control

*indicates p<0.05 compared to Accutane only

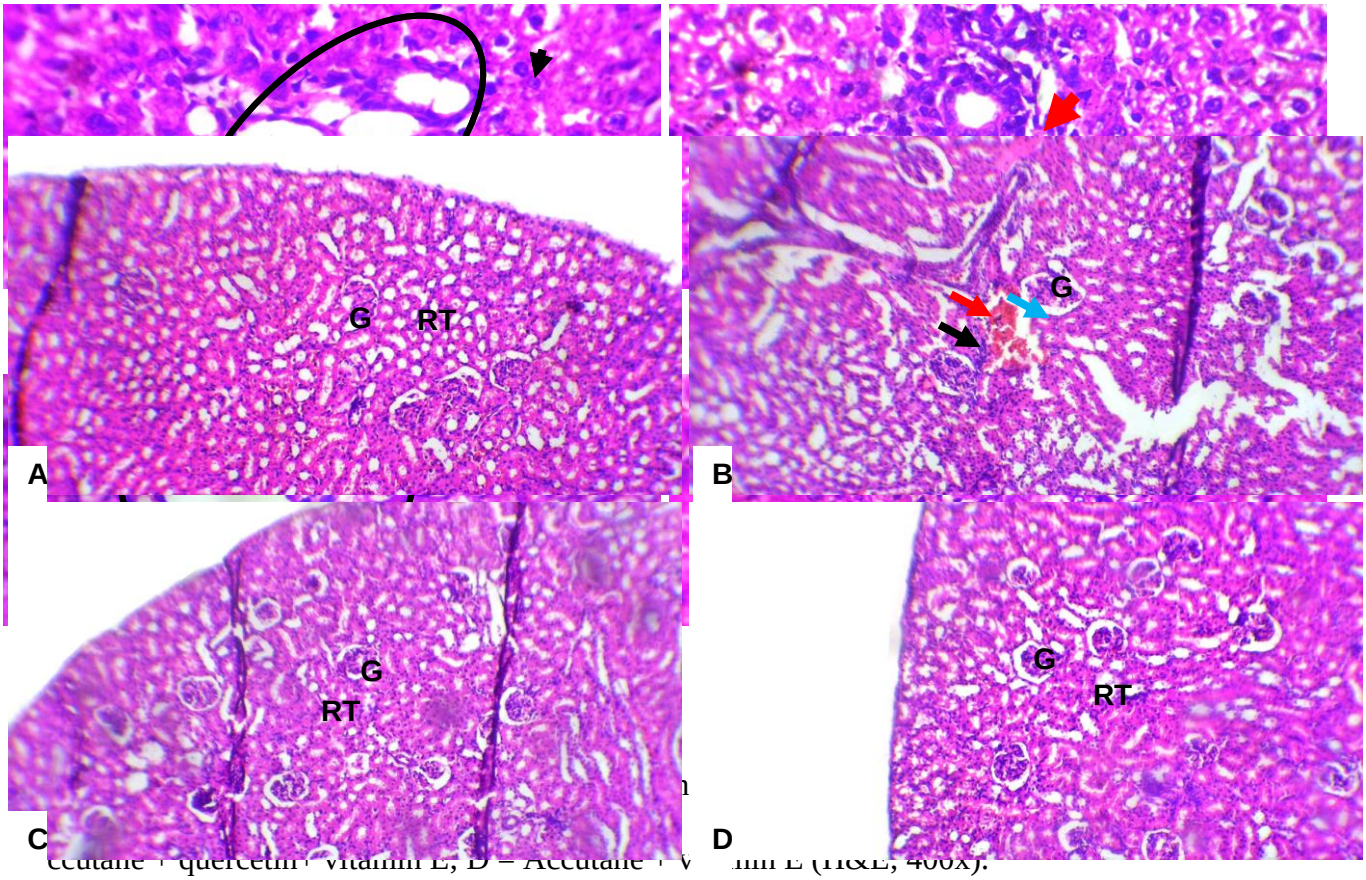
4.3 Histology Results of the Liver



Photomicrographs of liver of the experimental animals: A = control; B = Accutane; C = Accutane + quercetin+ vitamin E; D = Accutane + vitamin E (H&E; 100x).

Groups A and C show normal histological features: portal tract (encircled), hepatocytes (black arrows)

In group B, there is severe periportalinfiltrates of inflammatory cells (red arrow). In group D, there is mild periportalinfiltrates of inflammatory cells (red arrow).



Groups A and C show normal histological features: portal tract (encircled), hepatocytes (black arrows)

In group B, there is severe periportalinfiltrates of inflammatory cells (red arrow). In group D, there is mild periportalinfiltrates of inflammatory cells (red arrow).

4.4 Histology Results of the Kidneys

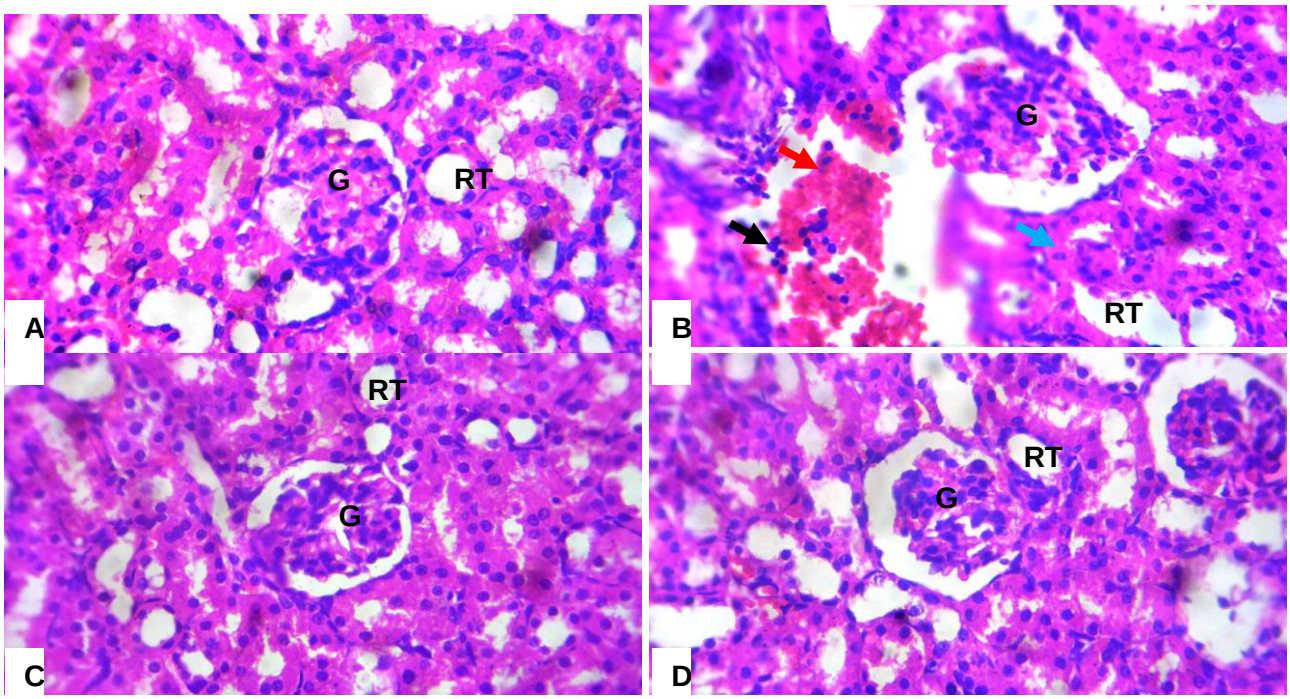


Plate 4.3: Representative histology at 100×

Photomicrographs of kidneys of the experimental animals: A = control; B = Accutane; C = Accutane + quercetin+ vitamin E; D = Accutane + vitamin E (H&E; 100x)

Groups A, C and D show normal histological features: glomerulus (G), renal tubules (RT)

In group B, there is severe interstitial congestion (red arrow), mild infiltrates of inflammatory cells (black arrow) and focal tubular swelling (blue arrow)

Plate 4.4: Representative histology at 400×

Photomicrographs of kidneys of the experimental animals: A = control; B = Accutane; C = Accutane + quercetin+ vitamin E; D = Accutane + vitamin E (H&E; 400x)

Groups A, C and D show normal histological features: glomerulus (G), renal tubules (RT)

In group B, there is severe interstitial congestion (red arrow), mild infiltrates of inflammatory cells (black arrow) and focal tubular swelling (blue arrow)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study examined the effects of Isotretinoin (Accutane) on fetal growth, hepatic and renal histology, and the possible protective influence of co-treatment with the antioxidants Vitamin E and Quercetin in a rat model. The findings clearly demonstrate that isotretinoin exposure during pregnancy exerts deleterious effects on fetal development and maternal organ histology, while antioxidant supplementation significantly mitigates these toxic impacts.

A significant reduction in fetal birth weight and crown-rump length was observed in the Accutane-only group compared to the control. This aligns with the established teratogenic and growth retarding potential of isotretinoin, a derivative of retinoic acid known to interfere with cellular proliferation, differentiation, and morphogenesis during embryonic development (Mitchell *et al*., 2019; Li *et al*., 2020). Retinoids are essential for normal morphogenesis, but excess levels can cause oxidative stress, disrupt normal gene expression, and trigger apoptosis in developing tissues (El-Hefnawy *et al*., 2021). The reduced fetal growth observed in this study may therefore be linked to oxidative imbalance and increased production of reactive oxygen species (ROS), leading to impaired placental function and nutrient transfer. Conversely, the group treated with Accutane + Vitamin E + Quercetin showed a significant improvement in crown-rump length and birth weight compared to the Accutane-only group. This suggests that combined antioxidant therapy alleviated the oxidative stress-induced developmental impairment. Previous studies have confirmed that antioxidants enhance fetal growth by restoring redox balance, stabilizing placental vasculature, and protecting against free radical mediated tissue damage (Kumar *et al*., 2021; Atessahin *et al*., 2006). Thus, the current findings indicate that Vitamin E and Quercetin, acting synergistically, preserved fetal health and promoted normal growth despite isotretinoin exposure.

Histological examination of the liver revealed that Accutane-only groups showed severe periportal inflammatory infiltrates, signifying hepatocellular injury and inflammation. The liver is a primary site of isotretinoin metabolism, and this biotransformation process can generate toxic intermediates that increase oxidative stress and lipid peroxidation (Daye *et al*., 2020). Reactive oxygen species attack polyunsaturated fatty acids in cellular membranes, leading to structural disruption and the release of pro-inflammatory mediators. This explains the marked periportal inflammation seen in the Accutane-only group. However, in animals co-treated with Vitamin E and Quercetin, the hepatic structure closely resembled the control group, with normal portal tracts and well-arranged hepatocytes. This observation indicates strong hepatoprotective potential of the antioxidants. Importantly, the Accutane + Vitamin E group showed mild periportal inflammation less severe than the Accutane-only group but not completely normal suggesting partial protection by Vitamin E. Vitamin E, being a lipid-soluble antioxidant, localizes within cellular membranes where it interrupts lipid peroxidation chains by donating hydrogen atoms to neutralize free radicals (Brigelius-Flohé & Traber, 2019). Its effect in this study implies that while Vitamin E mitigates oxidative injury, its protective efficiency increases when combined with Quercetin, which provides additional radical-scavenging and metal-chelating activity. The synergistic protection observed in the combined antioxidant group corroborates reports that flavonoids such as Quercetin can regenerate oxidized Vitamin E, enhancing its stability and prolonging its antioxidant activity (Boots *et al*., 2008; Saied *et al*., 2014). Therefore, the near-normal hepatic histology seen in the combination group strongly supports a complementary mechanism between both antioxidants one stabilizing lipid membranes (Vitamin E) and the other neutralizing ROS and modulating inflammatory signaling (Quercetin).

Kidney sections from the Accutane-only group revealed severe interstitial congestion, inflammatory cell infiltration, and focal tubular swelling. These findings indicate that

isotretinoin also exerts nephrotoxic effects, possibly through oxidative stress and inflammatory pathways. The kidney's high oxygen consumption and abundance of polyunsaturated lipids make it particularly vulnerable to ROS-induced injury (Türedi *et al*., 2020). Oxidative damage to renal tubular cells leads to mitochondrial dysfunction, impaired ion transport, and eventual necrosis or apoptosis. In contrast, the Accutane + Vitamin E and Accutane + Vitamin E + Quercetin groups exhibited near-normal renal histoarchitecture, similar to the control. This suggests that both antioxidants effectively preserved renal integrity, with the combination therapy providing optimal protection. Vitamin E likely reduced membrane lipid peroxidation, while Quercetin may have modulated pro-inflammatory cytokines and improved antioxidant enzyme activities (SOD, CAT, and GSH). These effects align with studies showing that antioxidant supplementation reduces renal oxidative injury and inflammation in drug-induced toxicity models (Nabavi *et al*., 2012; Öztürk *et al*., 2021).

The protective effects observed in both hepatic and renal tissues can be attributed to the combined antioxidant capacity of Vitamin E and Quercetin. Isotretinoin's toxic mechanisms are thought to involve excessive ROS generation, mitochondrial stress, and disruption of endogenous antioxidant systems. Vitamin E, by integrating into cell membranes, interrupts lipid peroxidation cascades and stabilizes structural integrity, whereas Quercetin acts both as a direct free radical scavenger and as an enhancer of endogenous antioxidant defenses (Liguori *et al*., 2018). Moreover, Quercetin modulates transcription factors like NF-κB and Nrf2, which control the expression of inflammatory and antioxidant genes, respectively. The synergy of these two antioxidants thus provides dual protection limiting oxidative damage and suppressing inflammatory responses. The finding that Vitamin E alone provided only partial protection in hepatic tissues while the combined therapy fully preserved histological architecture supports the hypothesis that multiple antioxidants acting through complementary mechanisms yield superior protective outcomes. This is consistent with earlier studies demonstrating enhanced

antioxidant efficacy from combined flavonoid and tocopherol treatment against xenobiotic-induced oxidative stress (Atessahin *et al*., 2006; Boots *et al*., 2008).

Overall, the present study highlights the dual organ toxicity potential of isotretinoin on the liver and kidneys, as well as the therapeutic advantage of antioxidant co-treatment. Since isotretinoin is still widely prescribed in dermatological therapy, understanding its systemic impact is clinically relevant. The findings suggest that oxidative stress is a central mediator of its hepatotoxic and nephrotoxic effects, and that supplementation with potent antioxidants such as Vitamin E and Quercetin could provide an effective protective strategy. Furthermore, the observed improvements in fetal parameters under antioxidant co-treatment emphasize the importance of redox balance during pregnancy. Antioxidant therapy may serve as a preventive approach against drug-induced teratogenicity, though further research is necessary to translate these findings into clinical contexts. In summary, isotretinoin induced marked hepatic and renal alterations characterized by periportal inflammation, interstitial congestion, and tubular swelling, while Vitamin E and Quercetin co-administration preserved normal histoarchitecture and improved fetal growth indices. These findings underscore the crucial role of oxidative stress in isotretinoin-induced toxicity and demonstrate that combination antioxidant therapy offers superior protection compared to single agent supplementation.

5.2 Conclusion

Findings from the study show that isotretinoin (Accutane) significantly impaired fetal growth and induced distinct histological damage in both hepatic and renal tissues in rats. Importantly, co-treatment with Vitamin E and Quercetin offered protection evidenced by improved organ histology and fetal growth parameters. The study also support the hypothesis that oxidative stress is an underlying mechanism of isotretinoin-induced toxicity, and that antioxidant therapy can mitigate these adverse effects. Therefore, the combination of Vitamin E and Quercetin

represents a promising protective strategy against retinoid‐induced organ toxicity and growth impairment.

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