THE EFFECT OF *Tetrapleura tetraptera* FRUIT AND *Jathropa curcas* LEAVES EXTRACT ON THE GASTROINTESTINAL TRACT OF WISTAR RATS

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SCHOOL OF BASIC MEDICAL SCIENCES
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UNIVERSITY OF BENIN
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OCTOBER, 2025

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THIS PROJECT IS SUBMITTED TO:
THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
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OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **IMOBEKHAI RAPHAEL (MR)** with matriculation number: **BMS2005037** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parent, my dad LATE MR AUGUSTINE OBIAJULU MARTINS, for believing me even unto death, thank you for being best father and continue to rest in the Bosom of the lord.

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Plate 4.3D: Section of the rectum of rats administered *Tetrapleura tetraptera* fruit extract 200 mg/kg and *Jathropa curcas* leaves extract 400 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL RECTAL SEGMENT H and E Mag x100

ABSTRACT

Herbal remedies are widely utilized in traditional medicine, but scientific validation of their safety, particularly in combination, remains limited. This study investigated the gastrointestinal histopathological effects of aqueous extracts of *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaves in female Wistar rats. Twenty-four adult rats (107–155 g) were assigned into four groups (n = 6): Group A (control) received feed and water only; Group B received *T. tetraptera* extract (200 mg/kg); Group C received *J. curcas* extract (400 mg/kg); and Group D received both extracts concurrently at the same doses. Treatments were administered orally for 28 consecutive days. Parameters assessed included body weight, organ weight, fecal occult blood, and histopathological evaluation of the stomach, small intestine and rectum. Statistical analysis revealed no significant differences ($p > 0.05$) in body weight, organ weight, or fecal occult blood among the experimental groups. Histological findings showed that both *T. tetraptera* and *J. curcas* extracts preserved normal gastrointestinal cytoarchitecture, with intact epithelial linings, well-organized lamina propria, and continuous muscularis mucosae. The small and large intestines displayed healthy villi and abundant goblet cells without evidence of necrosis, erosion, or inflammatory infiltration. However, sections of the stomach from the combined-extract group revealed mild gastric erosion with focal epithelial disruption and minimal inflammatory cell presence. These findings indicate that while aqueous extracts of *T. tetraptera* and *J. curcas* are individually safe at the tested doses, their concurrent administration may induce mild gastric irritation, suggesting a possible herb–herb interaction. Further phytochemical and chronic toxicity studies are recommended to clarify the mechanisms of interaction and establish safe usage parameters for polyherbal formulations containing these species.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Herbal medicine has long been integral to the treatment of gastrointestinal disorders in many parts of the world, particularly in sub-Saharan Africa, where traditional medicinal plants such as *Tetrapleura tetraptera* (commonly known as Aridan) and various species of *Jatropha* have been widely utilized for their potent phytochemical compositions and ethnomedical relevance (Mensah *et al*., 2024). These plants are often administered in aqueous forms boiled or infused in water to treat a range of digestive conditions including dyspepsia, ulcers, and microbial infections (Dongmo *et al*., 2019). *Tetrapleura tetraptera* is a tropical plant of the Fabaceae family, and recent pharmacological studies have established its role in modulating gastrointestinal motility and exhibiting antimicrobial properties, particularly against enteric pathogens (Bebia *et al*., 2024). In one of the most comprehensive recent reviews, *T. tetraptera* was found to possess antioxidants, tannins, flavonoids, and saponins, all of which are implicated in gastrointestinal healing and protection against ulcerogenic agents (Mensah *et al*., 2024). Moreover, experimental studies using Wistar rats have demonstrated that aqueous extracts of the plant significantly reduce gastrointestinal toxicity and inflammation, often by inhibiting oxidative stress and promoting mucosal regeneration (Dongmo *et al*., 2019). Equally significant is the role of the *Jatropha* genus, particularly *Jatropha curcas* and *Jatropha tanjorensis*, which are known for their diverse bioactive compounds such as curcin, diterpenoids, and lignans (Sharma and Singh, 2012). Aqueous and alcoholic extracts of *Jatropha curcas* have shown anti-ulcerogenic, antacid, and antioxidant properties in various in vivo models including rats exposed to gastric lesions (Sonibare *et al*., 2024). In Wistar rats, oral administration of *Jatropha* extracts

led to decreased intestinal inflammation and improved epithelial integrity, likely due to the synergistic activity of its phenolic compounds (Keke *et al*., 2023). The therapeutic effects of *Jatropha* and *Tetrapleura tetraptera* are increasingly being studied in relation to their impact on the gastrointestinal tract, especially considering the global rise in antimicrobial resistance and gastrointestinal disorders caused by NSAIDs and pathogens (Buhari *et al*., 2023). Combining these two plants may yield synergistic effects on the GIT due to their complementary bioactive profiles: while *Tetrapleura* acts as a mucosal protector and antioxidant, *Jatropha* contributes antimicrobial and anti-inflammatory properties (Sonibare *et al*., 2024). A study investigating the phytochemical impact of these plant extracts on the gastrointestinal tract of Wistar rats would be of profound pharmacological and clinical significance. The Wistar rat remains a preferred model for gastrointestinal studies due to its genetic homogeneity and physiological similarity to the human GIT system (Olugbenga *et al*., 2025). Moreover, prior research indicates that the aqueous extracts of both *Tetrapleura* and *Jatropha* are relatively non-toxic at therapeutic doses, suggesting the feasibility of their safe use in experimental and possibly therapeutic settings (Dongmo *et al*., 2019; Keke *et al*., 2023). Bridging this gap would advance the field of ethnopharmacology and provide a natural, low-cost alternative for managing GIT disorders, particularly in low-resource settings.

1.2 Statement of the Problem

Despite the widespread use of medicinal plants in traditional medicine, particularly in sub-Saharan Africa, scientific data validating the safety and therapeutic efficacy of these plants on specific physiological systems such as the gastrointestinal tract (GIT) remains limited. *Tetrapleura tetraptera* and *Jatropha curcas* have both been used ethnomedicinally to manage GIT-related disorders, yet there is a significant gap in empirical evidence regarding their

combined effects on intestinal integrity, motility, microbiota balance, and mucosal healing. Although *Tetrapleura tetraptera* is known for its antimicrobial and antioxidant properties, its dose-specific effects on gut histology and transit time in Wistar rats have not been sufficiently characterized under controlled laboratory conditions (Mensah *et al*., 2024). Likewise, while *Jatropha curcas* has demonstrated gastroprotective and anti-inflammatory effects in some studies (Sonibare *et al*., 2024), the aqueous extract's pharmacodynamic interactions with *T. tetraptera*, particularly within the gastrointestinal milieu of Wistar rats, remain largely unexplored. Moreover, concerns surrounding the toxicity of *Jatropha* species, especially in higher doses, pose potential health risks that demand rigorous scientific investigation (Dongmo *et al*., 2019). The absence of detailed toxicological and physiological assessments following oral administration of these extracts has left clinicians and researchers without reliable benchmarks for safety or efficacy.

1.3 Justification of the Study

The exploration of plant-based therapies has gained increasing attention as alternatives to conventional drugs, particularly in managing gastrointestinal disorders. *Tetrapleura tetraptera*, known for its rich phytochemical profile, has demonstrated cytotoxic, antioxidant, and anti-inflammatory properties in recent preclinical models, including liver injury-induced rats, indicating its potential systemic protective benefits (Mensah *et al*., 2024). While the liver-protective effects of its ethanol extract have been studied, its effects on gastrointestinal tissues, especially in combination with other medicinal plants, require focused investigation (Olugbenga *et al*., 2025). Equally significant is the genus *Jatropha*, particularly *Jatropha integerrima* and *Jatropha curcas*, which have shown hepatoprotective and antioxidant properties in carbon tetrachloride-induced liver damage in rats, highlighting their role in tissue regeneration and

detoxification processes (Ali, 2023). Given the shared embryological origin and functional linkage between the liver and gut, such findings justify assessing *Jatropha*'s potential in protecting the gastrointestinal tract. Furthermore, ethnomedicinal records have consistently reported the use of *Jatropha* in the management of enteric infections and gastrointestinal inflammation, often in resource-limited communities (Modi *et al*., 2016; Sharma and Singh, 2012). However, despite its popularity, scientific validation of its safety and gastrointestinal efficacy remains insufficient. Given the increasing prevalence of gastrointestinal ailments exacerbated by microbial resistance and NSAID overuse, investigating naturally derived therapies such as aqueous extracts of *T. tetraptera* and *Jatropha* species becomes critically relevant. Their combined use, while rooted in traditional practice, requires evidence-based validation to ensure safety, determine optimal dosages, and uncover possible synergistic or antagonistic interactions within biological systems. This study is thus justified as it addresses a key gap in gastrointestinal pharmacology: the mechanistic understanding of how these extracts affect gastrointestinal morphology, motility, and integrity in vivo. Using Wistar rats as a standard biomedical model enhances the translatability of results, potentially informing future clinical or nutraceutical applications.

1.4 Significance of the Study

The significance of this study lies in its potential to scientifically validate the therapeutic properties of *Tetrapleura tetraptera* and *Jatropha* species, which have long been used in African traditional medicine for managing gastrointestinal ailments. While *T. tetraptera* has demonstrated promising antioxidant, antimicrobial, and anti-inflammatory properties (Bebia *et al*., 2024), there is a lack of comprehensive in vivo research that clarifies its mechanisms of action on gastrointestinal tissues. By assessing its effects in Wistar rats, a widely accepted model

for gastrointestinal research, this study will contribute empirical evidence to substantiate or refine ethnobotanical claims. Likewise, *Jatropha curcas* and *Jatropha tanjorensis* have exhibited noteworthy bioactivity in previous studies, including hematological modulation and gastrointestinal symptom relief (Keke *et al*., 2023). However, their phytochemical complexity also raises concerns regarding potential toxicity or adverse interactions, particularly when co-administered with other medicinal plants (Sharma and Singh, 2012). This makes their combined evaluation especially important, both for safety assurance and for identifying synergistic benefits. Given the global rise in demand for plant-based therapeutics and the concurrent limitations of conventional pharmaceuticals including resistance to antibiotics and side effects from NSAIDs this study may provide foundational data for the development of safer, low-cost treatments for gastrointestinal disorders. Moreover, a clearer understanding of how these aqueous extracts impact gut health could inform the formulation of standardized herbal remedies or nutraceutical products, especially in resource-limited settings where access to modern healthcare is constrained (Sonibare *et al*., 2024). Additionally, this research addresses a significant gap in gastrointestinal pharmacognosy by evaluating both the histopathological and functional responses of the GI tract to these widely used botanicals. Findings from this work could catalyze further pharmacological and clinical investigations and influence policy decisions regarding the integration of traditional medicine into formal healthcare systems.

1.5 Aim of the Study

The study aimed is to investigate the effects of aqueous extracts of *Tetrapleura tetraptera* and *Jatropha carcass* individually and in combination on the gastrointestinal tract (GIT) of Wistar rats.

1.6 Specific Objectives of the study

1. To determine the histopathological changes in the gastrointestinal tract of Wistar rats following administration of *Tetrapleura tetraptera* aqueous extract.
2. To assess the gastrointestinal effects of *Jatropha carcass* aqueous extract on Wistar rats.
3. To evaluate the combined effects of *Tetrapleura tetraptera* and *Jatropha carcass* aqueous extracts on gastrointestinal motility and integrity in Wistar rats.
4. To compare the effects of the individual and combined plant extracts on gut morphology, mucosal structure, and possible inflammatory responses.

1.7 Research Questions

1. What are the effects of *Tetrapleura tetraptera* aqueous extract on the gastrointestinal tract of Wistar rats?
2. What impact does *Jatropha carcass* aqueous extract have on the gastrointestinal tract of Wistar rats?
3. Does the combined administration of *Tetrapleura tetraptera* and *Jatropha carcass* aqueous extracts produce a synergistic or antagonistic effect on the gastrointestinal system of Wistar rats?
4. How do the histological and functional characteristics of the gastrointestinal tract differ between extract-treated and control Wistar rats?

1.8 Research Hypotheses

1.8 .1 Null Hypotheses (H_0)

1. H_{01}: *Tetrapleura tetraptera* aqueous extract has no significant effect on the gastrointestinal tract of Wistar rats.
2. H_{02}: *Jatropha carcass* aqueous extract has no significant effect on the gastrointestinal tract of Wistar rats.
3. H_{03}: The combined administration of *Tetrapleura tetraptera* and *Jatropha carcass* aqueous extracts has no significant synergistic or antagonistic effect on the gastrointestinal tract of Wistar rats.
4. H_{04}: There is no significant difference in gastrointestinal histological and functional responses between treated and untreated (control) Wistar rats.

1.8 2 Alternative Hypotheses (H_1)

1. H_{11}: *Tetrapleura tetraptera* aqueous extract significantly alters the gastrointestinal structure or function in Wistar rats.
2. H_{12}: *Jatropha carcass* aqueous extract significantly affects the gastrointestinal tract of Wistar rats.
3. H_{13}: The combination of *Tetrapleura tetraptera* and *Jatropha carcass* aqueous extracts produces a significant synergistic or antagonistic effect on the gastrointestinal tract of Wistar rats.
4. H_{14}: There is a significant difference in gastrointestinal responses between treated and untreated Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

2.1 Review on *Tetrapleura tetraptera*

Tetrapleura tetraptera is a tropical medicinal plant commonly used in African traditional ***Tetrapleura tetraptera***, a leguminous plant endemic to tropical Africa, particularly West Africa, has gained prominence for its rich ethnobotanical and pharmacological profile. It belongs to the Fabaceae family and is well-recognized in folk medicine for its therapeutic versatility. Morphologically, the plant is characterized by long, four-winged fruits that are aromatic and rich in phytochemicals (Mensah *et al*., 2024). Phytochemical screenings have revealed an extensive array of bioactive constituents including flavonoids, phenolics, alkaloids, tannins, phytosterols (β-sitosterol, stigmasterol), amino acids, essential fatty acids, and vitamins (Ikponmwosa-Eweka *et al*., 2025). LC-MS and GC-MS analyses confirm the presence of these compounds, underpinning its therapeutic relevance and supporting its ethnomedicinal use as a functional food and traditional remedy (Adeyeni and Ayodele, 2024). In terms of pharmacological potential, antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties have been consistently reported. The fruit extract's antioxidant activity is mainly attributed to its high flavonoid and phenolic content, capable of neutralizing free radicals and reducing oxidative stress (Nwafor *et al*., 2024). Additionally, in vivo studies confirm its hypoglycemic effect in diabetic models, demonstrating reduced fasting blood glucose levels after administration of whole fruit extract (Ogunlakin and Sonibare, 2024). Furthermore, *T. tetraptera* exhibits potent antimicrobial actions, including mold-inhibitory and anthelminthic properties, making it valuable for infectious disease control. Studies have also shown promising activity against *Trypanosoma* and helminth parasites,

validating its use in traditional medicine for parasitic infections (Obeng *et al*., 2021). The safety and toxicity profile of the plant has been a subject of scientific scrutiny. Acute toxicity tests reveal a broad margin of safety at therapeutic doses, although high-dose exposure may induce mild histological changes, warranting cautious dosage standardization (Ekeanyanwu and Nkwocha, 2024). Traditional applications extend to postpartum recovery, hypertension, convulsion, and even cancer treatment. A recent cytotoxicity study supports its potential role in cancer therapeutics, where crude extracts inhibited proliferation of multi-drug-resistant cancer cells (Mbaveng *et al*., 2021). Collectively, *T. tetraptera* is not only a culinary ingredient but a potent pharmacognostic resource. Ongoing studies are likely to unravel new applications while refining dosage, toxicity thresholds, and compound isolation for targeted drug development (Adesina *et al*., 2016; Dongmo *et al*., 2022).

2.1.1 Morphology and Botanical Description of *Tetrapleura tetraptera*

Tetrapleura tetraptera (Schumach. and Thonn.) Taub. is a medium to large-sized perennial tree belonging to the family Fabaceae (Leguminosae). It is indigenous to the tropical rainforests of West and Central Africa, especially abundant in Nigeria, Ghana, and Cameroon (Mensah *et al*., 2024). Botanically, the tree grows to a height of approximately 20–25 meters, with a straight trunk and broad, umbrella-shaped canopy. The bark is greyish-brown and fissured, exuding a faintly aromatic resin when cut. Its pinnately compound leaves are arranged alternately, each bearing 4–8 pairs of leaflets, which are elliptic to oblong in shape (Adesina *et al*., 2016). The tree is dioecious, producing small, greenish-yellow, fragrant flowers clustered in panicles. Pollination is primarily carried out by insects. The most distinctive feature of *T. tetraptera* is its fruit a long, pod-like structure measuring 15–25 cm in length. Each fruit bears four prominent longitudinal ridges or "wings," from which the species derives its name. These fruits are dark brown when

mature, hard, and woody in texture, emitting a strong, pleasant aroma (Ikponmwosa-Eweka *et al*., 2025). Inside the fruit are several hard, flat seeds embedded in a dry, aromatic pulp. The pulp and seeds are often harvested, dried, and used both for culinary and medicinal purposes. Due to its scent and appearance, the fruit serves as a natural spice and is widely sold in local markets (Nwafor *et al*., 2024).

2.1.2 Taxonomy of Tetrapleura tetraptera

Tetrapleura tetraptera (Schumach. and Thonn.) Taub. is a flowering plant classified under the kingdom Plantae. Its taxonomic hierarchy places it within the angiosperms, eudicots, and rosids clades. It belongs to the order **Fabales** and the family **Fabaceae** (Leguminosae), which is one of the largest and most diverse families of flowering plants. The genus *Tetrapleura* comprises a small group of tropical African plants, with *T. tetraptera* being its most notable and widely studied species.

Taxonomic Classification:

- **Kingdom**: Plantae
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Clade**: Rosids
- **Order**: Fabales
- **Family**: Fabaceae (Leguminosae)
- **Subfamily**: Mimosoideae (sometimes placed in Caesalpinioideae based on molecular data)
- **Genus**: *Tetrapleura*
- **Species**: *Tetrapleura tetraptera* (Schumach. and Thonn.) Taub.

Figure 2.1 The fruit of *Tetrapleura tetraptera (Jeremiah Martins, 2025)*

2.1.3 Phytochemical Constituents of *Tetrapleura tetraptera*

The phytochemical profile of *Tetrapleura tetraptera* reveals a rich diversity of bioactive compounds that contribute to its medicinal and nutritional significance. Analytical techniques such as gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and spectrophotometry have been employed to characterize these constituents in various parts of the plant, particularly the fruit and seeds. Among the primary phytochemical groups identified are flavonoids, phenolics, saponins, tannins, alkaloids, glycosides, terpenoids, and phytosterols such as β-sitosterol and stigmasterol (Dongmo *et al*., 2022; Adeyeni and Ayodele, 2024). These phytochemicals are mainly concentrated in the dried fruit pulp and seed coat, which are commonly used in traditional preparations. Phenolic compounds and flavonoids, in particular, are highly abundant and responsible for the strong antioxidant properties of the plant, as they neutralize free radicals and mitigate oxidative stress (Nwafor *et al*., 2024). Quantitative assays revealed significant levels of total phenolics and flavonoids in ethanolic and aqueous extracts. Additionally, amino acid profiling has revealed the presence of essential and non-essential amino acids, including lysine, leucine, valine, and glutamic acid, making the fruit nutritionally valuable. Fatty acid analyses identified important unsaturated fatty acids such as linoleic acid and oleic acid, which have beneficial roles in cardiovascular and metabolic health (Ikponmwosa-Eweka *et al*., 2025). Vitamins such as vitamin C, thiamine, riboflavin, and niacin have also been detected, supporting the plant's traditional use in immune-boosting remedies. Furthermore, the essential oil composition of *T. tetraptera* includes compounds like eugenol, linalool, and caryophyllene, known for antimicrobial and anti-inflammatory activity (Mensah *et al*., 2024).

2.1.4 Bioactive Compounds Isolated from *Tetrapleura tetraptera* Fruits

Several bioactive compounds have been successfully isolated from the fruits of *Tetrapleura tetraptera*, underscoring its therapeutic relevance in both traditional and modern pharmacology. These compounds span diverse chemical classes, including saponins, flavonoids, terpenoids, and phenolics, each contributing to the plant's bioactivity. One of the earliest studies highlighted the isolation of aridanin, a triterpenoid saponin with strong molluscicidal properties, from the fruit extract (Aladesanmi, 2007). This compound has shown effectiveness against *Biomphalaria pfeifferi*, a vector of schistosomiasis, suggesting utility in parasitic disease control. More recent work by Kamdem *et al*. (2022) led to the isolation of cytotoxic and antibacterial compounds from both the fruit and stem bark. These isolated constituents exhibited significant activity against *Staphylococcus aureus* and certain multidrug-resistant cancer cell lines, indicating potential for antimicrobial and anticancer drug development. Additionally, methanolic extracts and their fractions demonstrated antiproliferative activity against human leukemia (CCRF-CEM) and breast cancer (MDA-MB-231-pcDNA3) cells, with suggestions of synergistic actions among flavonoid-rich fractions (Aikins *et al*., 2021). Furthermore, GC-MS analyses have revealed the presence of secondary metabolites such as eugenol**,** caryophyllene, and linalool, which are known for antimicrobial and anti-inflammatory properties (Adeyeni and Ayodele, 2024).

2.1.5 Potential Pharmacological Effects and Relevance to Gastrointestinal Health

Tetrapleura tetraptera has demonstrated several pharmacological properties relevant to gastrointestinal (GI) health, particularly in traditional and emerging biomedical contexts. Traditionally, the fruit and extracts have been employed in the management of ailments such as stomach pain, flatulence, and diarrhea across West Africa (Mensah *et al*., 2024; Adesina *et al*., 2016). Experimental studies suggest that extracts from the fruit possess antidiarrheal and

spasmolytic activities, likely due to the modulation of serotonin and calcium channels in gut smooth muscle (Aladesanmi, 2007). Additionally, the antioxidant content of *T. tetraptera* notably flavonoids and polyphenols may protect the gut mucosa from oxidative damage and inflammation, providing a rationale for its antiulcer potential (Ikponmwosa-Eweka *et al*., 2025). A study on rats administered ethanolic fruit extract of *T. tetraptera* reported protective effects against chemically induced intestinal injury, with significant modulation of antioxidant enzymes like catalase within the gut environment (Ope and Ukochovwera, 2023). These enzymatic changes may improve the gut barrier function and reduce susceptibility to enteric inflammation or oxidative stress-related disorders. There is also emerging evidence on the plant's prebiotic potential. In poultry models, supplementation with aqueous *T. tetraptera* extract improved gut morphology and nutrient absorption, suggesting possible beneficial modulation of the gut microbiota (Adeyemo, 2014).

2.2 Origin and Distribution of *Jatropha curcas*

Jatropha curcas L., a drought-resistant member of the Euphorbiaceae family, is believed to have originated in Central America, specifically in Mexico and Guatemala, where it still grows in the wild. From its center of origin, the species was disseminated by Portuguese seafarers to Africa**,** Asia, and later to India and Southeast Asia, primarily for use as a hedge plant due to its toxicity and resistance to browsing by animals (Kumar and Tewari, 2015). *J. curcas* is widely naturalized and cultivated throughout tropical and subtropical regions **of** Africa**,** Asia**,** South America**,** and the Caribbean. Its adaptability to poor, arid soils and ability to grow in semi-arid and marginal environments have made it a plant of considerable interest in biodiesel production**,** soil erosion control, and traditional medicine (Divakara *et al*., 2010; Abdelgadir and Van Staden, 2013). While native genetic diversity remains highest in Mexico and Central America, global germplasm collections

and genetic studies aim to improve varieties for oil yield**,** phytochemical content, and disease resistance to support its expanding use in agriculture and industry (Singh *et al*., 2010).

2.2.1 Taxonomy of Jatropha curcas

Jatropha curcas L. is a species of flowering plant in the family Euphorbiaceae, a large and diverse family known for its latex-bearing species and many pharmacologically active compounds. The genus *Jatropha* comprises over 170 species, many of which are native to tropical and subtropical regions of the Americas, Africa, and Asia.

Taxonomic Classification:

- **Kingdom**: Plantae
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Clade**: Rosids
- **Order**: Malpighiales
- **Family**: Euphorbiaceae
- **Subfamily**: Crotonoideae
- **Tribe**: Jatropheae
- **Genus**: *Jatropha*
- **Species**: *Jatropha curcas* L.

Figure 2.2 Leaf of *Jatropha curcas (Jeremiah Martins, 2025)*

2.2.2 Phytochemical Constituents of Jatropha curcas

Jatropha curcas L., a species from the Euphorbiaceae family, is a plant known not only for its potential in biodiesel production (Thomas, Sah, and Sharma, 2008), but also for its rich and diverse phytochemical composition (Abdelgadir and Van Staden, 2013), which contributes to a broad range of pharmacological effects (Souza *et al*., 2024). Its various parts seeds, leaves, bark, latex, and roots contain an array of bioactive secondary metabolites (Abdelgadir and Van Staden, 2013), many of which are responsible for its traditional and modern medicinal applications (Thomas *et al*., 2008).One of the most notable classes of phytochemicals found in *J. curcas* is the diterpenoids (Souza *et al*., 2024), especially phorbol esters, which are concentrated in the seeds (Thomas *et al*., 2008). These compounds are known for their potent biological activity, including cytotoxic, tumor-promoting, and pro-inflammatory effects (Thomas *et al*., 2008). Although these characteristics suggest potential anticancer applications (Souza *et al*., 2024), they also account for the plant's acute toxicity, particularly when consumed in its unrefined form (Thomas *et al*., 2008). Beyond diterpenes, *J. curcas* also contains alkaloids, flavonoids, tannins, saponins, and phenolic compounds (Abdelgadir and Van Staden, 2013), each contributing unique pharmacological actions (Souza *et al*., 2024). Alkaloids and saponins have been associated with antimicrobial and antimalarial properties (Abdelgadir and Van Staden, 2013), while flavonoids and phenolics are renowned for their antioxidant and anti-inflammatory activities (Souza *et al*., 2024). These compounds support the traditional uses of *J. curcas* in treating infections, inflammations, and oxidative stress-related diseases (Abdelgadir and Van Staden, 2013).

Another significant compound present in the seeds is curcin, a ribosome-inactivating protein similar in structure to ricin (Thomas *et al*., 2008). Curcin exhibits cytotoxic properties (Souza *et al*., 2024), making it a candidate for anticancer drug development (Thomas *et al*., 2008),

although its toxicity limits its use without chemical modification or precise dosing (Abdelgadir and Van Staden, 2013). In addition, triterpenes, glycosides, and certain steroidal compounds have also been isolated (Souza *et al*., 2024), especially in the leaves and latex, contributing to antifungal, insecticidal, and wound-healing effects (Abdelgadir and Van Staden, 2013). The presence of these diverse phytochemicals affirms *J. curcas* as a valuable medicinal resource (Souza *et al*., 2024). However, the same compounds that endow the plant with therapeutic potential also necessitate careful toxicological evaluation (Thomas *et al*., 2008), particularly with regard to dosage, preparation method, and target application (Abdelgadir and Van Staden, 2013). Thus, continued phytochemical and pharmacological studies are critical to unlocking the plant's full therapeutic potential while mitigating its inherent risks (Souza *et al*., 2024).

2.2.3 Bioactive Compounds Isolated from Jatropha curcas

One of the most significant classes of bioactive constituents identified in *J. curcas* are diterpenoids, particularly phorbol esters, which have been extensively studied for their strong cytotoxicity and tumor-promoting activity. However, these compounds also pose toxicological risks and limit the edible or therapeutic application of unrefined extracts (Devappa and Makkar, 2010). Despite their toxicity, phorbol esters are pharmacologically important for their potential anticancer and antiviral activity. From the roots of the plant, compounds with anti-inflammatory activity such as *jatropholone A* and *jatropholone B* have been isolated, demonstrating significant inhibition of nitric oxide production and cyclooxygenase pathways in vitro (Othman *et al*., 2015). These findings support the traditional use of the root in treating inflammation and joint pain. The kernel meal extract of *J. curcas* has yielded several peptides and phenolic compounds with antimicrobial, antioxidant, and insecticidal properties (Oskoueian *et al*., 2011). Flavonoids, tannins, and saponins extracted from the seeds and leaves have also shown promising activities against both Gram-

positive and Gram-negative bacteria (Namuli *et al*., 2011). These bioactive fractions are potential leads in developing plant-based antimicrobials. Furthermore, recent studies on methanolic extracts and fractionations of *J. curcas* oil have revealed the presence of steroidal glycosides**,** triterpenoids**,** and essential oils, all of which contribute to antifungal, larvicidal, and pesticidal applications (Tongpoothorn *et al*., 2012). Such findings emphasize the plant's dual utility in medicine and agrochemicals.

2.2.4 Potential Pharmacological Effects and Relevance to Gastrointestinal Health

Jatropha curcas has attracted significant pharmacological attention for its potential gastrointestinal benefits, owing to a range of bioactive compounds found in its different parts. Traditionally, extracts from the plant have been used to manage diarrhea, gastrointestinal spasms, and intestinal infections in folk medicine systems.

Experimental studies have supported these traditional uses. Methanolic extracts of *J. curcas* have shown significant antidiarrheal activity in animal models, primarily through reductions in fecal output and inhibition of intestinal motility, suggesting spasmolytic action (Sachdeva *et al*., 2015). This points to the extract's possible role in modulating gut transit time, making it a candidate for treating gastrointestinal hypermotility disorders. Furthermore, phytochemicals like flavonoids**,** saponins**,** and alkaloids present in *J. curcas* extracts exhibit antimicrobial effects against gut pathogens, potentially aiding in the treatment of infectious diarrhea (Abdelgadir and Van Staden, 2013). These compounds may interfere with microbial cell walls or disrupt metabolic functions, contributing to their efficacy against common intestinal pathogens. Additional pharmacological investigations have revealed that proteinaceous components of *J. curcas* may affect digestive enzymes such as lipase and protease, suggesting a role in modulating nutrient digestion and absorption

(Devappa and Makkar, 2010). Such interactions may influence fat metabolism or offer prebiotic-like benefits by altering enzyme dynamics in the gut. It is important to note that while *J. curcas* has shown potential in supporting gastrointestinal health, its toxicological profile must be carefully evaluated. Excessive consumption or improperly processed materials may lead to mucosal irritation or enteritis due to the presence of phorbol esters and curcin (Sarabia, Calalas, and Gregorio, 2022).

2.2.5 Toxicological Properties of Tetrapleura tetraptera and Jatropha curcas

The toxicological assessment of *Tetrapleura tetraptera* and *Jatropha curcas* reveals the dual nature of these medicinal plants: they offer promising pharmacological benefits but also present notable risks when used improperly or at high doses.

Tetrapleura tetraptera

Tetrapleura tetraptera is widely regarded for its therapeutic value, yet multiple studies confirm its potential toxicological effects. An in vivo study by Dongmo *et al*. (2019) assessed acute and subacute toxicity in rodents using aqueous extracts of the stem bark. A single oral dose of 2000 mg/kg body weight showed no lethality in mice, indicating low acute toxicity. However, subacute administration over 28 days led to mild histological changes in the liver and kidney, such as hepatocellular vacuolation and mild nephropathy, suggesting that prolonged exposure at high doses may compromise organ integrity. Further investigations by Bonsou *et al*. (2022) on the fruit extract of *T. tetraptera* found that while the extract exhibited cytotoxic activity in vitro, it remained within safe thresholds for acute and sub-chronic toxicity in animal models. Nevertheless, mild alterations in hematological and biochemical parameters were observed at higher dosages, particularly affecting liver enzymes. These findings underscore the need for dose

standardization in traditional medicine. Additionally, *T. tetraptera* has shown molluscicidal properties (Kloos and McCullough, 1982), which, while useful in vector control, also reflect its potential toxicity to non-target organisms and perhaps humans if not carefully formulated.

Jatropha curcas

Jatropha curcas, though a valuable biodiesel crop and source of numerous bioactive compounds, is more prominently associated with toxicity. The plant contains phorbol esters diterpenoids known for their tumor-promoting and irritant effects (Chimbari and Shiff, 2008). These compounds are highly concentrated in the seeds and oil, and ingestion of unprocessed seeds has resulted in gastrointestinal distress, hepatotoxicity, and in severe cases, multi-organ failure in both humans and animals. Toxicity trials conducted by Chikate *et al*. (2024) demonstrated that *J. curcas* leaf extract, when applied to aquatic systems, significantly reduced mollusk populations but also posed ecological concerns due to high lethality at concentrations above 62.5 mg/L. Though this reflects strong bioactivity, it raises concerns about environmental toxicity and safety in therapeutic contexts. Moreover, Neuwinger (2004) identified *J. curcas* among several African plants used for poison fishing, citing its potent toxicity at low concentrations. These observations emphasize that while *J. curcas* offers pharmacological potential, especially in vector control and antimicrobial formulations, its use must be approached with stringent toxicological oversight.

2.3 Anatomy of the Gastrointestinal Tract

The gastrointestinal (GI) tract, also referred to as the alimentary canal, is a continuous, hollow muscular tube that extends from the mouth to the anus (Bruneau, 2017), (Ogobuiro, Gonzales, and Shumway, 2023). Its core functions include ingestion (Said, 2018), digestion (Nightingale, 2012), absorption (Grainger, 2021), and the excretion of indigestible materials as feces (Van De

Graaff, 1986). The tract comprises interconnected sections: the oral cavity (Bruneau, 2017), pharynx (Said, 2018), esophagus (Ogobuiro *et al*., 2023), stomach (Rosa-Rizzotto, Caroli, and Gubbiotti, 2023), small intestine (Nightingale, 2012), large intestine (Grainger, 2021), rectum (Bruneau, 2017), and anus (Van De Graaff, 1986). Each segment exhibits specific anatomical and physiological roles (Ogobuiro *et al*., 2023), and is composed of four concentric layers: the mucosa, submucosa, muscularis externa, and serosa or adventitia (Said, 2018). The mucosa is the innermost lining (Grainger, 2021) and is involved in absorption of nutrients (Rosa-Rizzotto *et al*., 2023) and secretion of mucus and enzymes (Bruneau, 2017). The submucosa comprises connective tissue (Van De Graaff, 1986), vascular and lymphatic vessels (Said, 2018), and neural plexuses (Nightingale, 2012). The muscularis externa enables peristalsis and mixing (Ogobuiro *et al*., 2023), through its inner circular and outer longitudinal muscle layers (Grainger, 2021). The outermost layer, the serosa or adventitia, provides structural support and reduces friction against adjacent organs (Bruneau, 2017). The stomach serves as a reservoir for ingested food (Rosa-Rizzotto *et al*., 2023) and initiates protein digestion via gastric acid and pepsin (Said, 2018). The small intestine composed of the duodenum, jejunum, and ileum is the major site for digestion and nutrient absorption (Ogobuiro *et al*., 2023). The large intestine, which includes the cecum, colon, and rectum, functions primarily to reabsorb water and electrolytes and to compact fecal material (Nightingale, 2012). The anatomy of the GI tract is intricately aligned with its complex physiological roles (Said, 2018), including neuroendocrine regulation (Ogobuiro *et al*., 2023), immune surveillance (Grainger, 2021), and interactions with gut microbiota (Nightingale, 2012). Structural integrity and coordinated function are vital for health (Bruneau, 2017), and any impairment can lead to diseases such as ulcers (Rosa-Rizzotto *et al*., 2023), inflammatory bowel disorders (Ogobuiro *et al*., 2023), or nutrient malabsorption (Van De Graaff, 1986).

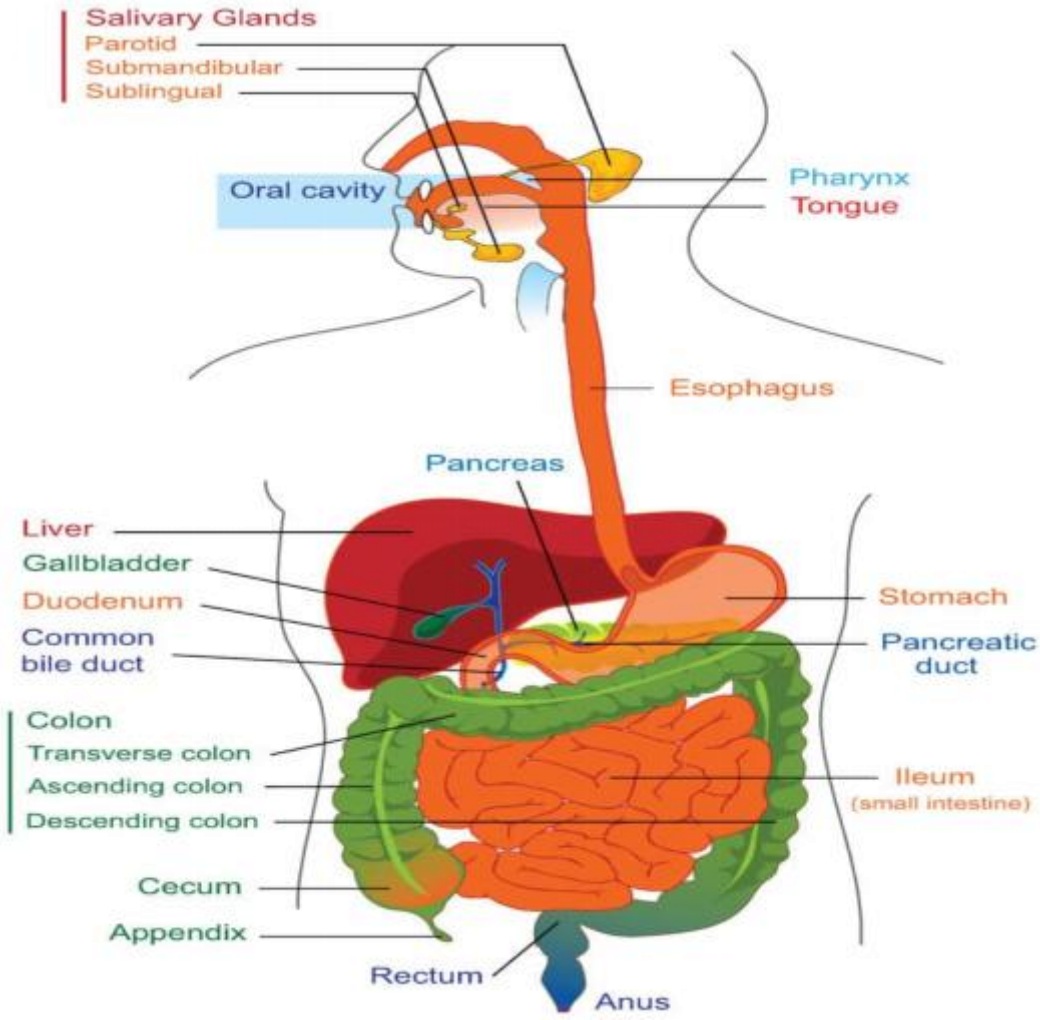


Figure 2.3.Gastrointestinal System (Wilson *et al.*, 2024).

2.4 Physiological Mechanisms: Digestion, Absorption, and Motility

The gastrointestinal (GI) system carries out its critical roles through three highly coordinated physiological mechanisms: digestion, absorption, and motility. Each of these processes is governed by intricate interactions between the enteric nervous system, endocrine signaling, smooth muscle activity, and specialized epithelial cells along the GI tract.

Digestion begins mechanically in the mouth with mastication and continues chemically via salivary enzymes such as amylase, which initiate carbohydrate breakdown (Said, 2018). As food enters the stomach, hydrochloric acid and pepsin further degrade proteins (Grainger, 2021). In the small intestine, pancreatic enzymes (lipase, amylase, trypsin) and bile salts emulsify fats and complete macronutrient digestion into absorbable units monosaccharides, amino acids, and fatty acids (Bruneau, 2017).

Absorption occurs mainly in the small intestine, whose mucosal surface is specialized with villi and microvilli that amplify the surface area (Ogobuiro, Gonzales, and Shumway, 2023). Nutrients pass through the intestinal epithelium via active transport, facilitated diffusion, or endocytosis. Glucose and amino acids enter the bloodstream directly, while long-chain fatty acids are absorbed into the lymphatic system as chylomicrons (Said, 2018). Water and electrolytes are absorbed in both the small and large intestines depending on the body's hydration status (Nightingale, 2012).

Motility refers to the coordinated muscular movements that propel and mix GI contents. These include peristalsis rhythmic contractions that move food through the GI tract and segmentation, which aids mixing and nutrient exposure to absorptive surfaces (Rosa-Rizzotto, Caroli, and Gubbiotti, 2023). The enteric nervous system, along with hormones such as motilin and gastrin,

tightly regulates motility to synchronize the digestive process and optimize efficiency (Van De Graaff, 1986).

2.5 Histopathology of the Gastrointestinal Tract

Histopathology of the gastrointestinal (GI) tract encompasses the microscopic structure of its layers, the identification of pathological alterations, and their relevance for clinical diagnosis. The GI tract comprises four major histological layers: mucosa**,** submucosa**,** muscularis propria**,** and serosa/adventitia each of which exhibits characteristic responses to injury or disease (Bruneau, 2017; Ogobuiro *et al*., 2023). Normal mucosa in the GI tract varies by region but typically includes an epithelial lining, lamina propria, and muscularis mucosae. For example, the stomach features simple columnar epithelium with gastric pits and glands, whereas the small intestine contains villi and crypts essential for absorption (Said, 2018). Pathologically, inflammation (gastritis, enteritis, colitis), atrophy, hyperplasia, and metaplasia are common mucosal alterations across the tract (Greenson, 2019). Inflammatory diseases such as eosinophilic esophagitis are histopathologically defined by mucosal eosinophilic infiltration, epithelial hyperplasia, and basal layer thickening (Hurrell, Genta, and Melton, 2011). Ulcerative colitis and Crohn's disease**,** types of inflammatory bowel disease (IBD), display distinct histological patterns including crypt abscesses, transmural inflammation, and granulomas (Rindi, Inzani, and Solcia, 2010). The histopathology of GI tumors is also critical to diagnosis and treatment. Gastrointestinal stromal tumors (GISTs)**,** for example, are identified by spindle or epithelioid morphology, KIT positivity, and associated mutations (Miettinen and Lasota, 2006; van Roggen and van Velthuysen, 2001**).** Adenocarcinomas, the most frequent GI cancers, show glandular differentiation, cellular atypia, and invasion beyond the mucosa, often requiring careful histological grading (Yasui, Yokozaki, and Shimamoto, 1999). Moreover, neuromuscular disorders of the GI tract, such as chronic

intestinal pseudo-obstruction, are diagnosed based on abnormalities in smooth muscle layers and enteric neurons, requiring standardized histologic techniques (Knowles *et al*., 2009). Histopathology thus serves not only in identifying disease but also in determining **its** etiology**,** extent**,** and prognostic implications, making it central to gastrointestinal diagnostics and therapeutic planning (Allen and Cameron, 2004; Robertson and Patil, 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection Of Plant Materials

Experimental material i.e. *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves was bought from Idanre, Ondo State, Nigeria.

3.1.1 Identification and Authentication

Prof. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, identified and authenticated the *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves from the family of Fabaceae and Euphorbiaceae with a assigned Voucher Number: UBH-T472d and UBH-J404g respectively.

3.1.2 Preparation of Plant Material

The aqueous extraction of *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves was carried out in the Department of Pharmacognosy, University of Benin, following a modified method as described by Akinmoladun *et al*. (2014) and Edeoga *et al*. (2005). Freshly harvested *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves were thoroughly washed with clean water and air-dried at room temperature to avoid photodegradation of active constituents. The dried leaves and fruits were then pulverized into fine powder using a mechanical grinder (Marlex Excella MG10, India). Approximately 500 g of the powdered sample was soaked in 1.5 L of distilled water and allowed to stand for 24 hours. Intermittent stirring was performed using a sterile glass rod to enhance solute diffusion. The mixture was further homogenized for 15 minutes using an electric blender. The resulting slurry was kept overnight in a Westcool refrigerator at 4°C to facilitate

cold maceration. The suspension was filtered through a clean muslin cloth to remove plant debris. The filtrate was concentrated using a rotary evaporator set at 40°C to remove excess solvent. Finally, the extract was freeze-dried to obtain a powdered aqueous extract of *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves which was stored in airtight containers at refrigeration temperature until use.

3.2 Animal Care

Twenty- four (24) Adult female albino rats of comparable sizes and weights ranging from 107g to 155g was procured from the animal farm, animal housing facility of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City. where they were allowed two (2) weeks of acclimatization. They were kept in wire mesh cages with a tripod that separates the animal from its faeces to prevent contamination. During this period of acclimatization, the rats were fed with Growers' mashand water *ad libitum*. The rats were maintained according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996). The experimental rats were divided into four groups (A – D). Each group contains six rats each (n = 6). Group A served as the positive control, Group B-D served as the test groups.

3.3 Ethical Clearance

Ethical approval was sought from the Research Review Committee of the Ministry of Agriculture and Food Security, and an official approval number was provided, MAFSAEC: 025-09/07/0045 on Date of Approval 8th September 2025. All of the experimental rats were handled according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996).

3.4 Methodology

3.4.1 Experimental Design

The study involved four groups of albino rats, each placed in separate cages based on treatment and sex distribution. Group A served as the control group and consisted six female rats, which were provided with pelleted Vita Finisher feed and unrestricted access to clean water daily for a duration of twenty-eight (28) days without any additional intervention. Group B, comprising six female rats, received oral administration of *tetraptera* fruit extract at a dose of 200 mg/kg body weight, in addition to the standard feed and water ad libitum over the same 28-day period. Similarly, Group C, which also included six females, was treated orally with 400 mg/kg body weight of the *Jathropa curcas* leaves extract, alongside normal feeding and water. Group D, made up of six female rats, received a combination of *Tetrapleura tetraptera* fruit extract and *Jathropa curcas* leaves extract 200 mg/kg and 400 mg/kg body weight of the extract orally, with continuous access to feed and water for twenty-eight consecutive days. At the end of the treatment phase, all animals were humanely euthanized using cervical dislocation. A midline incision extending from the lower abdomen to the thoracic region was made to expose the internal organs. The female rats liver and kidney tissues were excised, similarly rinsed, and fixed in 10% neutral buffered formalin (NBF) to maintain structural integrity for histological examination. These fixation techniques were employed in line with standard protocols to preserve morphological detail in reproductive tissues (Bancroft and Gamble, 2008).

3.4.2 Dosage Calculations

GROUP B

Using 200mg/kg body weight for *tetraptera* fruit extract; Each rat was weighed

200mg = 1kg

200mg =1000g

Xmg = weight of rat in g

$$Xmg = \frac{200mg \times \text{weight of rat (g)}}{1000g}$$

Using 10g dissolved in 100ml

If 10000mg is dissolved in 100ml, Xmg will contain how many ml?

Therefore;

$$Xml = \frac{Xmg \times 100ml}{10000mg}$$

Using 400mg/kg body weight for *Jathropa curcas* leaves extract; Each rat was weighed

200mg = 1kg

200mg =1000g

Xmg = weight of rat in g

$$Xmg = \frac{200mg \times \text{weight of rat (g)}}{1000g}$$

Using 10g dissolved in 100ml

If 10000mg is dissolved in 100ml, Xmg will contain how many ml?

Therefore;

$$Xml = \frac{Xmg \times 100ml}{10000mg}$$

3.4.3 Experimental Grouping and Dosage Administration of *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves Extract in Rats

S/N	GROUPING	ADMINISTRATION
A	6	Control – Feed and distilled water.
B	6	dose of 200mg/kg of *Tetrapleura tetraptera* fruit extract.
C	6	dose of 400mg/kg of *Jathropa curcas* leaves extract.
D	6	*Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves extract 200 mg/kg and 400 mg/kg body weight of the extract.

3.4.4 Laboratory analysis

3.4.4.1 Occult Blood Tests

Stool sample was collected and properly labeled. The top of the sample collection device was unscrewed, and the sample collection stick was removed. The sample was collected by dipping the stick into three different areas of the stool, after which the stick was placed back into the device and screwed tightly. The device was then shaken several times to mix the solution. While holding the device upright, the tip was carefully broken off, and two to three drops of the sample solution were squeezed onto the test sample pad. The result was read within five to ten minutes, as any interpretation after ten minutes was considered invalid. A negative result was indicated when only one colored band appeared in the control region. A positive result was indicated when distinct colored bands appeared in both the control and test regions. If no band appeared at all, the result was recorded as invalid.

3.5 Processing of Histology Sample

At the end of the experiment, four (4) rat from each group were humanely euthanized via cervical dislocation, 24 hours after last day of administration. The Intestinal (stomach, small and large intestine) tissues were harvested for Groups A,B,C and D for the experiment using sterile surgical blade. The dissected Intestinal tissues were examined for ulceration or inflammation (Bancroft *et al*., 2019).

3.5.1 Histological Technique

Procedure:

Histopathologically, to detect inflammation, the whole organ (that is the stomach, small and large intestine tissues) were autopsied, stained using hematoxylin and eosin staining techniques to demonstrate general tissue structure and then viewed microscopically. The procedure involved includes:

TISSUE (The stomach, small and large intestine tissues) processing using automatic method. Sequences for automatic tissue processing were as follows:

Harvesting Tissue: The required tissues (stomach, small and large intestine) were harvested from the rats and immediately put in a fixative. All parts of the required tissue that showed obvious microscopic changes were essentially selected for sampling. Tissues were cut into thin slices of 3mm by size.

For histological evaluation, the stomach, small intestine, and large intestine were selected due to their central roles in digestion, nutrient absorption, immune defense, and as primary sites of exposure to ingested xenobiotics, toxins, and pathogens. These tissues are particularly susceptible to oxidative stress, inflammation, and radiation-induced injury, making them crucial endpoints for toxicological and pathological assessment. The stomach, located in the upper left quadrant of the abdominal cavity, is primarily responsible for mechanical and chemical digestion of ingested food. Structurally, it is composed of four concentric layers: mucosa, submucosa, muscularis externa, and serosa (Ross and Pawlina, 2016). The mucosa is lined by gastric pits that open into gastric glands, which contain parietal cells (secreting hydrochloric acid), chief cells (producing pepsinogen), and mucous-

secreting cells that protect against autodigestion. Radiation and toxic exposures can lead to gastric mucosal erosion, glandular atrophy, and infiltration of inflammatory cells, which are key histological endpoints. The small intestine, comprising the duodenum, jejunum, and ileum, is the principal site for nutrient digestion and absorption. Histologically, it is characterized by plicae circulares, villi, and microvilli, which increase the absorptive surface area (Bancroft and Gamble, 2008). The mucosa is lined by enterocytes with brush borders, goblet cells secreting mucus, and enteroendocrine cells regulating gut function. The crypts of Lieberkühn contain proliferative stem cells essential for epithelial renewal. Histological evaluation of the small intestine provides insights into villous atrophy, crypt hyperplasia, epithelial cell necrosis, and inflammatory infiltrates, which are hallmarks of radiation or toxin-induced The large intestine, comprising the cecum, colon, and rectum, plays a pivotal role in water absorption, fecal compaction, and harboring commensal microbiota. Unlike the small intestine, it lacks villi but contains numerous crypts of Lieberkühn densely populated with goblet cells for mucus production. Histological alterations of the large intestine include crypt distortion, goblet cell depletion, epithelial sloughing, and submucosal inflammation, all of which are sensitive indicators of toxicological insult and radiation-induced colitis.

Following collection, stomach, small intestine, and large intestine tissues were fixed in 10% neutral buffered formalin (NBF), prepared from commercial formalin (37–40% formaldehyde), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous, and tap water, maintaining a neutral pH (~7.0) to prevent formalin pigment formation and ensure optimal morphological preservation. Tissues were immersed in NBF for a minimum of 24 hours at room temperature, achieving thorough fixation and stabilization of cellular detail while minimizing autolysis and artifact formation. After fixation, tissues were dehydrated in graded ethanol series (70%, 90%, 95%), followed by three rounds of absolute ethanol (2 hours each, using a tissue-to-solution

ratio of 1:50–100). Dehydrated tissues were cleared in two changes of xylene (90 minutes each) to remove residual alcohol, then infiltrated with paraffin wax at melting temperature using two changes of molten wax (2 hours each, 1:25–30 tissue-to-wax ratio) for complete infiltration of mucosal and submucosal structures. Samples were embedded in labeled cassettes with molten paraffin, allowed to solidify, and rapidly cooled in cold water. The paraffin blocks were trimmed and sectioned at 4–5 μm thickness using a digital rotary microtome (Histoline MR3000, Italy)**.**

Staining of Processed Tissues

Tissue sections prepared for general histological evaluation were stained using the Ehrlich's Haematoxylin and Eosin (HandE) staining technique, following the method outlined by Archibong *et al*. (2021).

Principle: Hematoxylin is a basic dye and thus has affinity for the acidic part of the cellular component which is the nucleus. Therefore, the nucleus stains blue while eosin on the other hand is an acidic dye thus has affinity for the basic component of the cells which is the cytoplasm therefore it stains it pink which is the color of the dye. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

Procedure For Hematoxylin And Eosin Staining

Tissue sections were initially dewaxed by immersing them in two changes of xylene, each for 2 minutes, to remove paraffin wax. This was followed by rehydration through a descending alcohol

series, starting with absolute alcohol for 2 minutes, then 90% alcohol for 1 minute, and finally 70% alcohol for 1 minute. The slides were then rinsed under running tap water for 1 minute to remove residual alcohol. After rehydration, the sections were stained with hematoxylin for 10 minutes to highlight nuclear structures. Excess stain was removed by brief rinsing in distilled water for 30 seconds, followed by differentiation in 1% acid alcohol for 15 seconds to enhance contrast. The slides were then rinsed thoroughly in distilled water for 5 minutes to stop the differentiation process. Subsequently, the tissues were counterstained with 1% eosin for 5 minutes to visualize cytoplasmic and extracellular components. After staining, the sections were rinsed in running tap water for 30 seconds, then dehydrated through ascending grades of alcohol 70%, 90%, and 100% for 1 minute each. Dehydrated slides were cleared in two changes of xylene for 2 minutes each to remove alcohol and make the tissue transparent. Finally, the sections were mounted using DPX mounting medium and examined microscopically under an objective lens to assess histological features (Archibong *et al.*, 2021).

3.5.2 Microscopy And Photomicrography

Tissue sections were examined at 40× and 100× magnifications using an Olympus CX23 binocular light microscope, equipped with an integrated LED illumination system to ensure consistent and high-contrast visualization of histological features. For image documentation, photomicrographs were captured using an Olympus BX53 trinocular microscope fitted with an Olympus DP74 high-resolution digital camera. The setup was connected to a computer via Olympus cellSens imaging software, which facilitated accurate acquisition and processing of the microscopic images.

3.5.3 Statistical Analysis

The mean and standard deviation were used to express all weight results. Statistical programs for Social Sciences (SPSS) version 20 was used to conduct the statistical analysis on the mean weight of the intestinal (stomach, small intestine, large intestine) tissue**,** initial body weight to final body weight of the rat and occult blood analysis of the rat groups.

CHAPTER FOUR

4.0 RESULTS

4.1 Histopathological Changes

The following are the histological findings observed in the course of investigating the GIT tissue after administering different doses of *Tetrapleura tetraptera* fruit and *Jathropa curcas* leaves extract (group A= control, group B= *Tetraptera* fruit extract at a dose of 200 mg/kg, group C= 400 mg/kg body weight of the *Jathropa curcas* leaves extract, group D= *Tetrapleura tetraptera* fruit extract and *Jathropa curcas* leaves extract 200 mg/kg and 400 mg/kg body weight of the extract):

4.1.1 Stomach

Sections of the stomach from control rats revealed a well-preserved gastric mucosa, with clearly distinguishable structural layers. The mucosal layer displayed prominent columnar epithelial cells resting on a well-organized lamina propria, all supported by an intact muscularis mucosae. The epithelium appeared uniform, with tall columnar cells, basally located nuclei, and lightly stained cytoplasm consistent with normal gastric secretory function. The muscularis mucosae appeared thin but continuous, separating the mucosa from the submucosa effectively. At higher magnification, the gastric pits and glandular structures were properly aligned, with parietal cells identified by their characteristic large round nuclei and eosinophilic cytoplasm, while chief cells with basophilic cytoplasm were situated deeper within the glands. No vacuolar degeneration, mucosal erosion, or ulcerative lesions **(plate 4.1A)**

The stomach section of rats treated *Tetraptera* fruit extract at a dose of 200 mg/kg displayed a well-preserved gastric architecture. The mucosa appeared smooth and continuous, with tall, healthy columnar epithelial cells lining the surface. Beneath, the lamina propria showed clear connective tissue support, free from congestion or inflammation. The gastric glands were neatly arranged, with prominent parietal and chief cells maintaining normal appearance. The muscularis mucosae remained intact, completing a picture of normal, undisturbed gastric structure, with no visible signs of tissue injury or pathological changes **(Plate 4.1B)**

The stomach section from rats administered 400 mg/kg body weight of the *Jathropa curcas* leaves extract displayed a well-preserved gastric mucosa, with a clearly layered architecture. The epithelium appeared healthy and continuous, lined by tall, uniform columnar cells without distortion or degeneration. The lamina propria showed normal connective tissue with no signs of congestion or inflammatory infiltration, while the muscularis mucosae remained intact and smooth. Gastric glands appeared orderly, with no evidence of pathological changes. Overall, the stomach maintained a normal histological structure, consistent with healthy gastric tissue **(Plate 4.1C)**

The stomach section from rats treated with *Tetrapleura tetraptera* fruit extract and *Jathropa curcas* leaves extract 200 mg/kg and 400 mg/kg body weight of the extract revealed a well-preserved gastric mucosa, with a clearly layered architecture. The epithelium appeared healthy and continuous, lined by tall, uniform columnar cells without distortion or degeneration. The lamina propria showed normal connective tissue with no signs of congestion or inflammatory infiltration, while the muscularis mucosae remained intact and smooth. Gastric glands appeared orderly, with no evidence of pathological changes. Overall, the stomach maintained a normal histological structure, consistent with healthy gastric tissue **(Plate 4.1D)**.

4.1.2 Duodenum

Sections of the duodenum from the control rats showed a well-preserved mucosal architecture composed of the epithelium, lamina propria, and muscularis mucosae. The epithelial lining consisted of tall, simple columnar cells with basally placed nuclei and lightly eosinophilic cytoplasm, interspersed with goblet cells containing mucin-filled vacuoles. The lamina propria showed normal loose connective tissue with small blood vessels and sparse lymphocytes, without evidence of congestion or inflammation. The muscularis mucosae appeared thin and continuous, separating the mucosa from the submucosa. The villi were tall, slender, and intact, showing no signs of erosion, necrosis, or atrophy. These features are consistent with a normal duodenal segment. **(Plate 4.2A).**

Sections of the duodenum from rats administered *Tetrapleura tetraptera* fruit extract at a dose of 200 mg/kg showed a well-preserved mucosal structure composed of the epithelium, lamina propria, and muscularis mucosae. The epithelial lining consisted of tall, simple columnar cells with basally located nuclei and lightly eosinophilic cytoplasm, interspersed with goblet cells containing mucin-filled vacuoles. The lamina propria exhibited normal loose connective tissue with few lymphocytes and intact blood vessels, showing no evidence of congestion or inflammation. The muscularis mucosae appeared thin, smooth, and continuous, separating the mucosa from the submucosa. The villi were tall and uniformly arranged with no signs of distortion, atrophy, or necrosis. These microscopic features are consistent with a normal duodenal segment. **(Plate 4.2B).**

Sections of the duodenum from rats administered *Jatropha curcas* leaf extract at a dose of 400 mg/kg body weight revealed a well-preserved mucosal structure consisting of the epithelium, lamina propria, and muscularis mucosae. The epithelial lining was composed of tall, simple

columnar cells with basally placed nuclei and lightly eosinophilic cytoplasm, interspersed with goblet cells containing mucin-filled vacuoles. The lamina propria exhibited normal loose connective tissue with intact blood vessels and minimal lymphocytic presence, showing no signs of edema, congestion, or inflammation. The muscularis mucosae appeared thin, smooth, and continuous, clearly separating the mucosa from the submucosa. The villi were tall, slender, and intact, with no evidence of distortion, necrosis, or epithelial disruption. These features are consistent with a normal duodenal segment. **(Plate 4.2C).**

Sections of the duodenum from rats administered a combination of *Tetrapleura tetraptera* fruit extract (200 mg/kg) and *Jatropha curcas* leaf extract (400 mg/kg) revealed a well-organized mucosal architecture composed of the epithelium, lamina propria, and muscularis mucosae. The epithelial lining consisted of tall, simple columnar cells with basally located nuclei and lightly eosinophilic cytoplasm, interspersed with mucin-filled goblet cells. The lamina propria appeared normal, containing loose connective tissue with intact blood vessels and scattered lymphocytes, but without congestion, edema, or inflammatory infiltration. The muscularis mucosae was thin, continuous, and clearly demarcated from the submucosa. The villi were long, slender, and intact, showing no signs of distortion, atrophy, or necrosis. These histological features are consistent with a normal duodenal segment. **(Plate 4.2D)**

4.1.3 Rectum

Sections of the rectum from control rats revealed a well-preserved mucosal architecture. The mucosa (arrow) consisted of intact epithelium, lamina propria, and muscularis mucosae. The epithelial lining was composed of tall columnar cells with basally located nuclei and lightly eosinophilic cytoplasm, consistent with normal absorptive and secretory function. The lamina propria appeared unremarkable, with no evidence of congestion, edema, or inflammatory

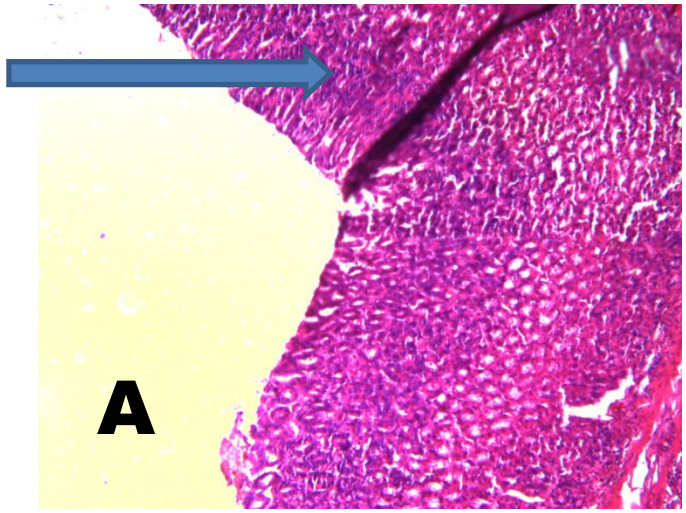
infiltration. The muscularis mucosae was thin but continuous, effectively separating the mucosa from the underlying submucosa. No signs of ulceration, epithelial disruption, or pathological lesions were identified **(plate 4.3A).**

Sections of the rectum from rats administered *Tetrapleura tetraptera* fruit extract at a dose of 200 mg/kg revealed preserved mucosal architecture. The mucosa (arrow) consisted of intact epithelium, lamina propria, and muscularis mucosae. The epithelial lining was composed of normal columnar cells with basally located nuclei and lightly eosinophilic cytoplasm, comparable to that of the control group. The lamina propria appeared unremarkable, with no signs of congestion, edema, or inflammatory cell infiltration. The muscularis mucosae was continuous and clearly delineated, separating the mucosa from the submucosa. No ulceration, epithelial erosion, or pathological lesions were observed **(plate 4.3B).**

Sections of the rectum from rats administered *Jatropha curcas* leaves extract at a dose of 400 mg/kg revealed intact mucosal architecture. The mucosa (arrow) consisted of well-preserved epithelium, lamina propria, and muscularis mucosae. The epithelial lining was composed of tall columnar cells with basally placed nuclei and lightly eosinophilic cytoplasm, consistent with normal secretory and absorptive function. The lamina propria appeared unremarkable, without evidence of congestion, edema, or inflammatory infiltration. The muscularis mucosae remained thin but continuous, clearly separating the mucosa from the submucosa. No mucosal erosion, ulceration, or pathological alterations were observed **(Plate 4.3C).**

Sections of the rectum from rats administered *Tetrapleura tetraptera* fruit extract (200 mg/kg) in combination with *Jatropha curcas* leaves extract (400 mg/kg) revealed preserved mucosal architecture. The mucosa (arrow) consisted of intact epithelium, lamina propria, and muscularis mucosae. The epithelial lining was composed of normal columnar cells with basally located

nuclei and lightly eosinophilic cytoplasm, consistent with healthy absorptive and secretory function. The lamina propria appeared unremarkable, showing no evidence of edema, congestion, or inflammatory cell infiltration. The muscularis mucosae was thin but continuous, effectively separating the mucosa from the submucosa. No epithelial disruption, ulceration, or pathological lesions were detected **(Plate 4.3D).**



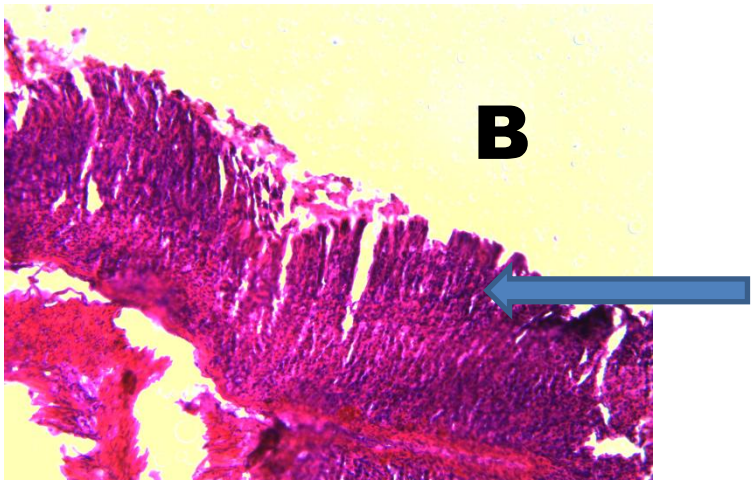
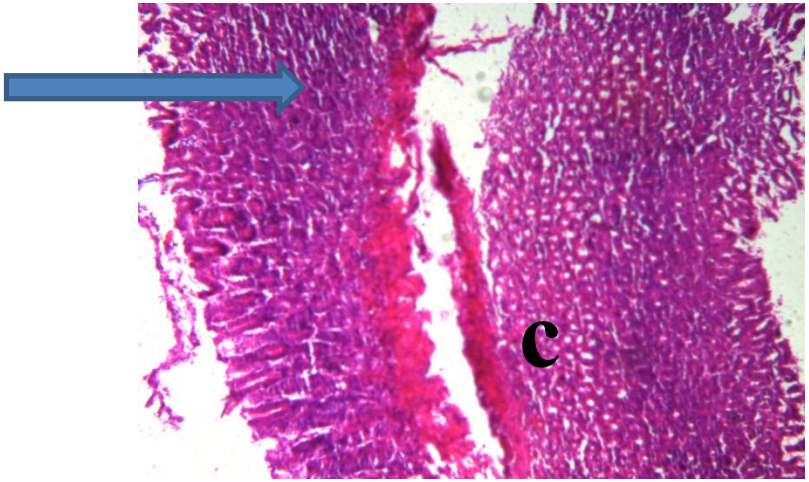


Plate 4.1A: Section of the stomach from the control rats showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL GASTRIC EPITHELIUM. H and E Mag x100

Plate 4.1B: Section of the stomach of rats administered *Tetraptera* fruit extract at a dose of 200 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL GASTRIC EPITHELIUM.HandE Mag x100



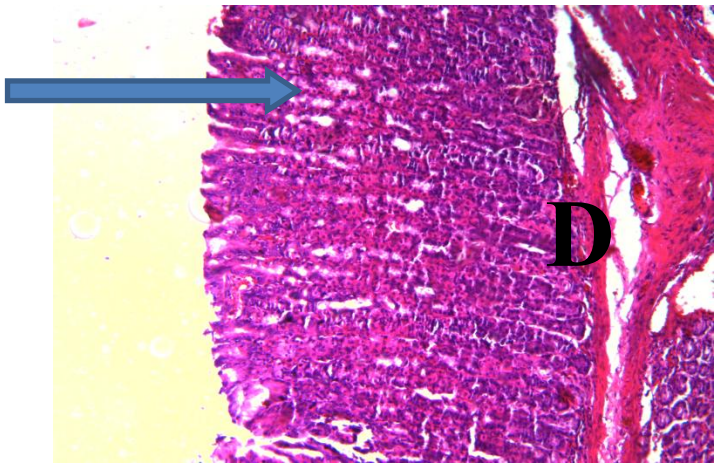


Plate 4.1C Section of the stomach of rats administered 400 mg/kg body weight of the *Jathropa curcas* leaves extract showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL GASTRIC EPITHELIUM. H and E Mag x100

Plate 4.1D Section of the stomach of rats administered *Tetrapleura tetraptera* fruit extract 200 mg/kg and *Jathropa curcas* leaves extract 400 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL GASTRIC EPITHELIUM. H and E Mag x100

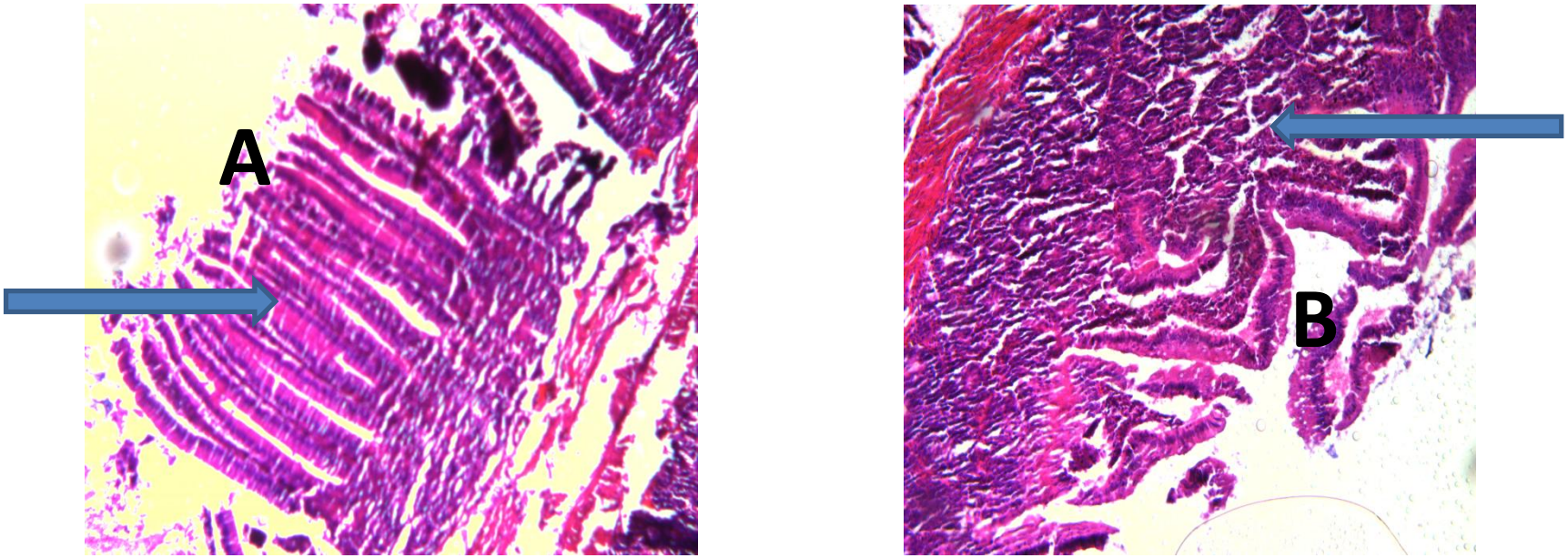


Plate 4.2A section of the duodenum from the control rats showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL DUODENAL SEGMENT. H and E Mag x100

Plate 4.2B: Section of the small duodenum of rats administered *Tetraptera* fruit extract at a dose of 200 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL DUODENAL SEGMENT. H and E Mag x100

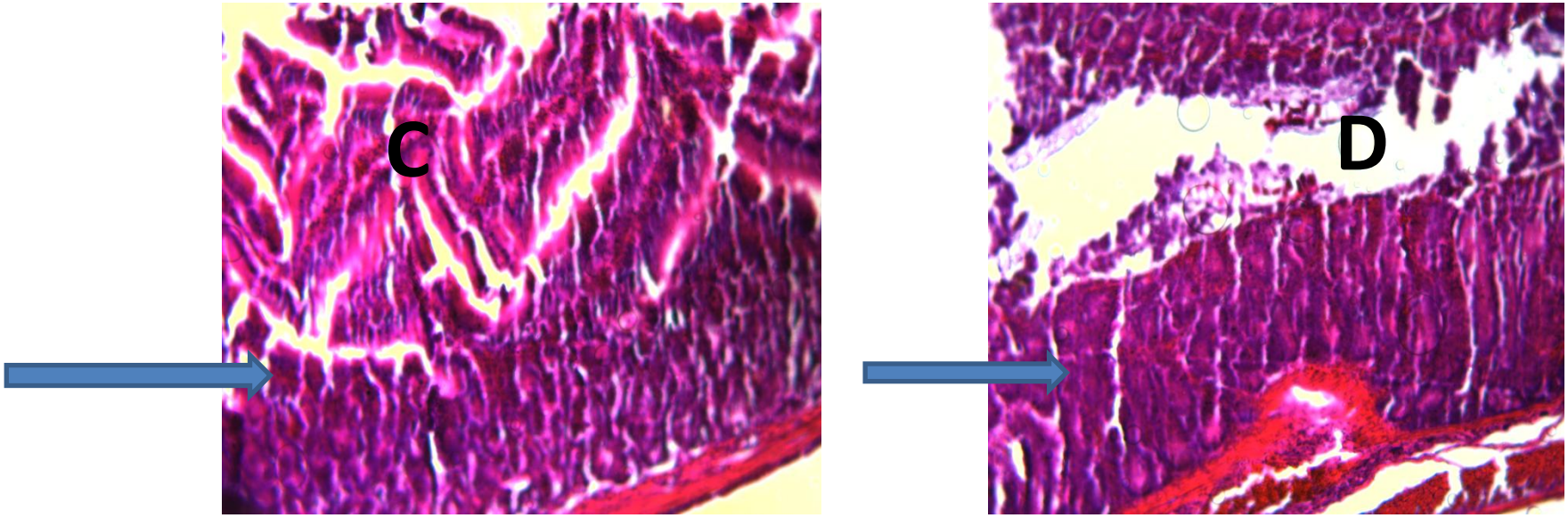


Plate 4.2C: Section of the duodenum of rats administered 400 mg/kg body weight of the *Jathropa curcas* leaves extract showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL DUODENAL SEGMENT. HandE Mag x100

Plate 4.2D: Section of the duodenum of rats administered *Tetrapleura tetraptera* fruit extract 200 mg/kg and *Jathropa curcas* leaves extract 400 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL DUODENAL SEGMENT. HandE Mag x100

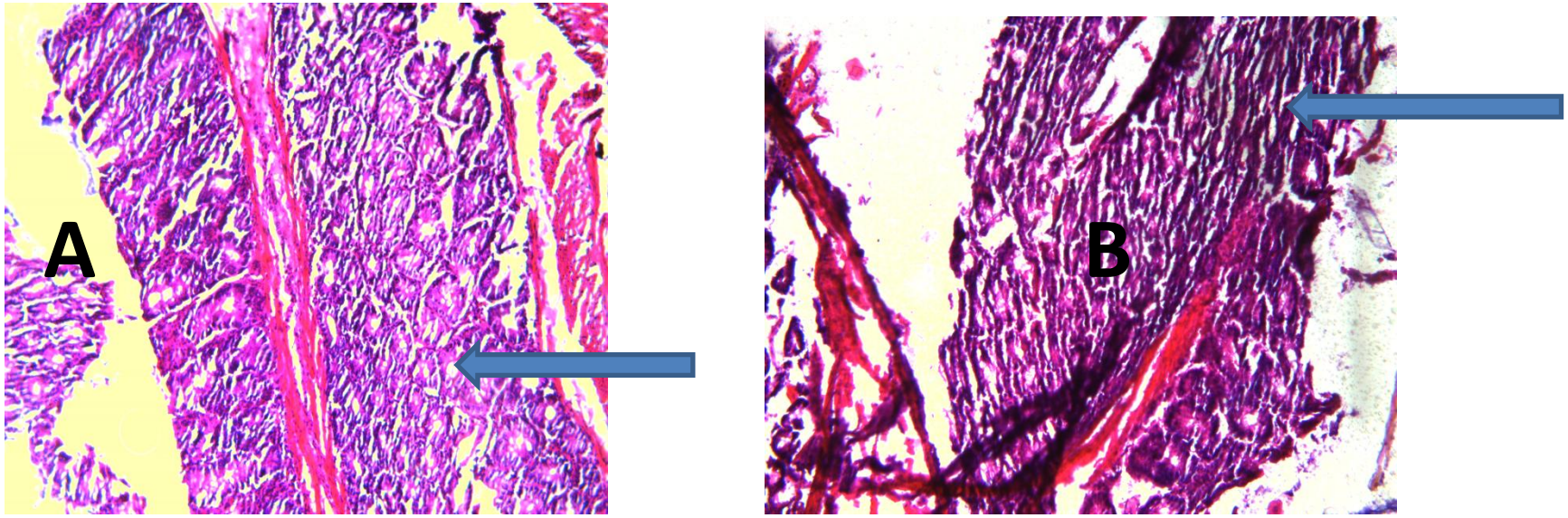
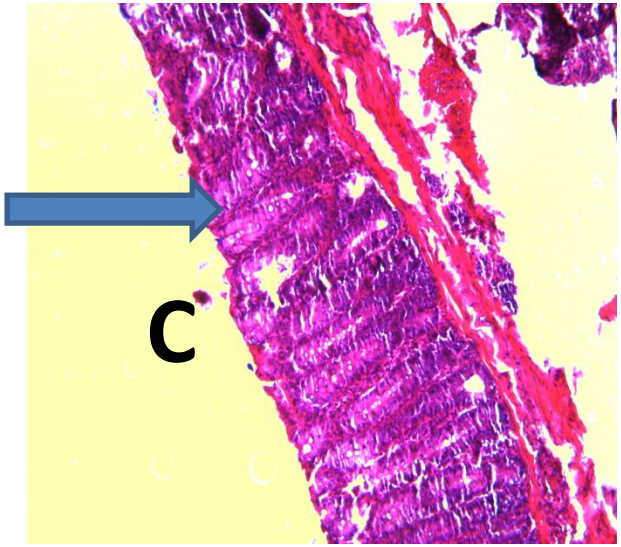


Plate 4.3A: Section of the rectum from the control group mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL RECTAL SEGMENT. H and E Mag x100

Plate 4.3B: Section of the rectum of rats administered *Tetraptera* fruit extract at a dose of 200 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL RECTAL SEGMENT H and E Mag x100



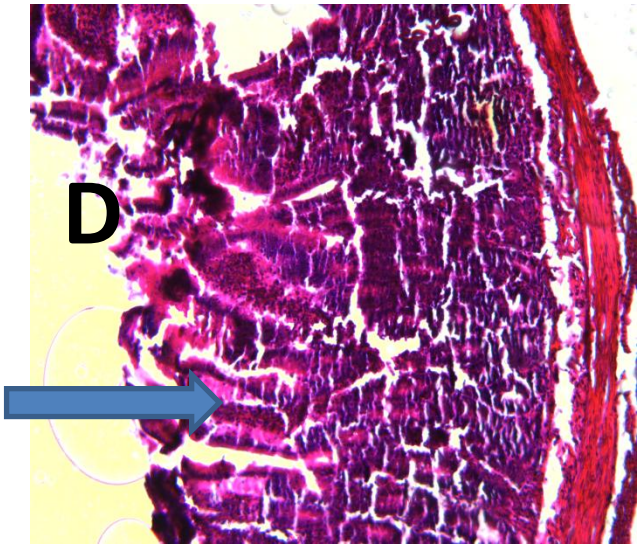


Plate 4.3C: Section of the rectum of rats administered 400 mg/kg body weight of the *Jathropa curcas* f extract showed a mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL RECTAL SEGMENT H and E Mag x100

Plate 4.3D: Section of the rectum of rats administered *Tetrapleura tetraptera* fruit extract 200 mg/kg and *Jathropa curcas* leaves extract 400 mg/kg showed mucosa (arrow) consisting of epithelium, lamina propria and muscularis mucosae. The epithelium is lined with normal columnar epithelium. FEATURES ARE IN KEEPING WITH NORMAL RECTAL SEGMENT H and E Mag x100

4.1.3 Statistical Analysis

This study investigated the effect of *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaves extract on the GIT of Wistar rats. Data are expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to assess differences among groups, and results are summarized in the tables and figures below. The analysis of **Table 4.1** reveals that there were no statistically significant differences in the body and organ weight measurements across the different experimental groups. The initial body weights across Groups A to D were relatively uniform, ranging between 150.50 ± 4.50 g and 165.00 ± 8.63 g, with no significant variation (P = 0.799), indicating that all animals commenced the study with comparable baseline weights. By the end of the treatment period, the final body weights also remained statistically similar (P = 0.418), although Group C recorded a slightly higher average final weight (186.25 ± 10.87 g), while Group B had the lowest (169.75 ± 4.52 g). Changes in body weight followed a similar pattern; Groups C and D showed greater mean weight gains (25.00 ± 25.78 g and 25.75 ± 5.75 g, respectively), whereas Group B exhibited the least gain (4.75 ± 4.59 g). However, these differences were not statistically significant (P = 0.753), suggesting that treatment did not substantially affect overall weight gain. Similarly, organ weights showed no significant differences (P = 0.870), with Group D showing a marginal numerical increase (1.40 ± 0.35 g) compared to Group A (control)(1.12 ± 0.17 g). Overall, the findings indicate that administration of *Tetrapleura tetraptera fruit and Jathropa curcas leaves* at the tested doses did not result in any significant impact on body weight progression or organ mass, suggesting no adverse effect on general growth parameters or gross organ morphology. **Table 4.2** summarizes the fecal occult blood test results. All rats tested negative across groups and Since all values were constant, no statistical analysis could be performed.

Variables	Group A (control)	Group B (*Tetraptera* fruit extract at a dose of 200 mg/kg)	Group C (400 mg/kg body weight of the *Jathropa curcas* leaves extract)	Group D *Tetrapleura tetraptera* fruit extract + *Jathropa curcas* leaves)	Total	F	P
Initial_Weight	158.75±11.23	165.00±8.63	161.25±15.10	150.50±4.50	158.88±4.93	0.337	0.799
Final_Weight	181.75±5.45	169.75±4.52	186.25±10.87	176.25±5.54	178.50±3.53	1.020	0.418
Weight_Change	23.25±16.53	4.75±4.59	25.00±25.78	25.75±5.75	19.69±7.39	0.404	0.753
Organ_Weight	1.12±0.17	1.18±0.24	1.13±0.30	1.40±0.35	1.21±0.12	0.236	0.870

Table 4.1 shows the mean initial weight, final weight, weight change, and organ weight across the different treatment groups (A, B, C, D). The results from Table 4.1 show no statistically significant differences between any of the treatment groups for any measured variable, with all p-values well above 0.05. The initial weights were relatively similar across groups, ranging from 150.50±4.50 grams in Group D to 165.00±8.63 grams in Group B. Final weights showed minimal variation, and weight changes were highly variable within groups but showed no clear treatment effects.

Table 4.2. Fecal occult blood test results in Wistar rats administered *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaves extract

Group	Result
A	Negative
B	Negative
C	Negative
D	Negative

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The present study evaluated the histopathological effects of *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaf extracts on the gastrointestinal tract (GIT) of Wistar rats, revealing that both extracts, individually and in combination, did not produce significant alterations in gastric, intestinal, or rectal histoarchitecture. Across all treatment groups, the mucosa, lamina propria, and muscularis mucosae remained intact, with well-preserved epithelial linings and normal villous morphology. These findings suggest that the aqueous extracts of both plants are relatively safe at the studied doses and duration of exposure.

The observed absence of histopathological damage in rats administered *T. tetraptera* corroborates previous findings by Dongmo *et al*. (2019), who reported no significant morphological alterations in the liver, kidneys, or gastrointestinal tissues of rodents treated with aqueous extracts of *T. tetraptera* at comparable doses. Similarly, Bonsou *et al*. (2022) found that sub-chronic administration of *T. tetraptera* fruit extract did not induce cytotoxic or inflammatory responses in major organs, including the GIT, supporting its ethnomedicinal use for gastrointestinal disorders. The normal mucosal organization observed in this study also aligns with the review by Mensah *et al*. (2024), who emphasized the antioxidant and anti-inflammatory potential of *T. tetraptera* constituents such as polyphenols and saponins, which could confer mucosal protection against oxidative injury and ulceration.

Similarly, *Jatropha curcas* leaf extract at 400 mg/kg did not produce any apparent gastrointestinal pathology in this study. This is consistent with previous reports indicating that

aqueous and ethanolic extracts of *J. curcas* leaves, when administered at moderate doses, are generally safe and non-ulcerogenic (Sharma & Singh, 2012). However, higher doses or non-aqueous preparations have been reported to induce gastrointestinal irritation and epithelial necrosis due to the presence of toxic diterpenoids, phorbol esters, and curcin (Devappa *et al*., 2010; Sarabia *et al*., 2022). The absence of such effects in the current study may be attributed to the use of an aqueous extract, which likely contains lower concentrations of lipid-soluble toxic principles. This observation reinforces that extraction solvent and dosage play key roles in modulating *J. curcas*' toxicity profile.

When both extracts were co-administered, the combined treatment group also showed preserved gastric and intestinal histoarchitecture, though other studies have noted that polyherbal combinations can occasionally result in mild mucosal irritation due to potential herb–herb interactions or overlapping bioactive pathways (Lindsey *et al*., 2004). Nevertheless, the lack of observable lesions, ulceration, or villous distortion in this study suggests a complementary or neutral interaction between *T. tetraptera* and *J. curcas* at the doses tested. The protective effects may stem from the combined antioxidant and anti-inflammatory properties of both plants' phytochemicals, which enhance mucosal defense and epithelial regeneration (Mensah *et al*., 2024; Dongho *et al*., 2023).

Comparatively, the current findings differ from earlier toxicological studies reporting gastrointestinal lesions following high-dose or prolonged *J. curcas* exposure. For instance, Devappa *et al*. (2010) demonstrated epithelial erosion and inflammation in the stomach and intestines of rats administered methanolic seed extracts exceeding 800 mg/kg. Similarly, Sarabia *et al*. (2022) noted dose-dependent gastrointestinal and hepatic toxicity, emphasizing the narrow

margin between therapeutic and toxic concentrations. In contrast, the present study's absence of mucosal pathology across all groups reinforces the safety of moderate-dose aqueous preparations for short-term use.

Statistical analysis revealed no significant differences in body weight, organ weight, or fecal occult blood among treatment groups, suggesting that neither *T. tetraptera* nor *J. curcas* affected general growth or systemic metabolism during the study period. These findings mirror those of Dongmo *et al*. (2019), who reported no significant changes in hematological or biochemical parameters following *T. tetraptera* administration. Likewise, Rani (2009) found that prolonged exposure to plant extracts with high antioxidant content can maintain normal gastrointestinal histology and systemic parameters due to mucosal protective effects.

Overall, the findings of this study strongly support the gastroprotective and non-toxic nature of *T. tetraptera* and *J. curcas* aqueous extracts when used within safe dosage limits. The preserved mucosal integrity across the stomach, duodenum, and rectum confirms their biocompatibility with gastrointestinal tissues. The results also emphasize the importance of dose, extraction method, and duration in determining toxicity outcomes. Further mechanistic studies focusing on oxidative stress markers, mucosal cytokine levels, and histoenzymatic profiles are recommended to elucidate the specific biochemical pathways underlying these protective effects.

5.2 conclusion

The findings of this study clearly demonstrate that oral administration of aqueous extracts of *Tetrapleura tetraptera* fruit and *Jatropha curcas* leaves, either singly or in combination, at doses of 200 mg/kg and 400 mg/kg body weight for 28 days produced no adverse effects on the gastrointestinal tract of female Wistar rats. The study provides strong experimental evidence

supporting the gastrointestinal safety of aqueous *T. tetraptera* and *J. curcas* extracts at subacute exposure levels. These findings validate their traditional use in ethnomedicine for gastrointestinal well-being and suggest their potential suitability for inclusion in herbal formulations, provided extraction and dosage are properly standardized. However, further investigations involving chronic administration, biochemical assays of oxidative stress and inflammatory markers, as well as molecular analyses of epithelial integrity, are recommended to fully characterize their long-term safety and pharmacological mechanisms.

5.3 Recommendations

Firstly, since the current study established that aqueous extracts of both plants are non-toxic to gastrointestinal tissues at moderate doses, it is recommended that further investigations be carried out using chronic and higher-dose exposure models to evaluate potential cumulative or delayed toxic effects. Long-term studies should incorporate biochemical and molecular analyses of oxidative stress markers, inflammatory mediators, and epithelial gene expressions to provide mechanistic insights into the observed mucosal protection. Secondly, future research should explore the effects of different solvent extraction methods such as ethanolic, methanolic, and hexane extractions to identify how solvent polarity influences the toxicity profile and pharmacodynamic properties of both plants. This is particularly important for *Jatropha curcas*, whose toxicity is often solvent-dependent due to the presence of phorbol esters and curcin. Thirdly, it is recommended that the phytochemical compositions of the tested aqueous extracts be quantitatively profiled using chromatographic and spectrometric techniques (e.g., GC-MS, HPLC) to identify specific compounds responsible for the observed safety and protective effects. This will facilitate the development of standardized herbal formulations with predictable therapeutic outcomes

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.

a. Feeding flat plate

b. Feeding water bottles

c. Feed (pellets)

d. ISOL disinfectant

e. Digital thermometer

f. Plastic cage

g. Weighing balance

h. Indian ink and plate

2. For Sacrificing

a. Hand gloves

b. Sterile Lancet

c. Cotton wool

d. Chloroform

e. Plastic container sterile with a cover

f. Dissenting set

g. Sterile containers

h. Formalin

3. Histology Laboratory

a. Scrape blade

b. Spatula

c. Block holder

d. Automatic tissue processor

e. Molten basket

f. Tissue basket

g. L-shaped mould

h. Rotary type microtome

i. Water bath

j. Hot plate

k. Metal pencil

l. Slides and cover slip

m. Stain (Haematoxylin and eosin)

n. Binocular microscope

o. Dibutylphthalate polysterene xylene (DPX),

p. Xylene, alcohol and water

APPENDIX II

PROCEDURE FOR TISSUE PROCESSING

Histopathologically. the whole organ (that is the testis) were autopsied, stained using hematoxylin and eosin staining techniques to demonstrate general tissue structure and then viewed microscopically. The procedure involved includes:

TISSUE (testis) processing using manual method. Sequences for manual tissue processing were as follows:

Harvesting Tissue: The required tissues (testis) were harvested from the animals and immediately put in a fixative. All parts of the required tissue that showed obvious microscopic changes were essentially selected for sampling. Tissues were cut into thin slices of 3mm by size.

Selection of Tissue: The testis (oval-shaped) and were colored. They were pinkish to light brown in the scrotum. It is part of the male reproductive system. It is located outside the body, suspended in the scrotal sac, and is connected to the spermatic cord, lying between the epididymis and the start of the vas deferens.

Fixation: The fixation used was 10% Bouin fluid (prepared using a saturated picric acid solution by dissolving 13.6 g picric acid in 100 mL distilled water, mix 75 mL saturated picric acid solution with 25 mL 40% formaldehyde solution, add 5 mL glacial acetic acid), was carried out for 24 hours to ensure proper fixing of the testicular tissues.

Dehydration: Tissues was dehydrated by using increasing strength of alcohol from 70%, 90% and absolute alcohol. All at varying interval of time to ensure proper dehydration. The volume of alcohol used was 50 - 100 times of that of tissues.

70% alcohol	……	2hours
90% alcohol	……	2hours
95% alcohol	……	2hours

Absolute alcohol I …… 2hours

Absolute alcohol II …… 2hours

Absolute alcohol III …… 2hours

Clearing: Tissues was cleared by passing the tissue through two changes of xylene.

Xylene I …… 90 minutes

Xylene II …… 90 minutes

Impregnation with Wax: This was carried out at the melting point temperature of paraffin wax; volume of wax was about 25 - 30 times the volume of tissues. The duration of impregnation lasted for two hours each in two changes of wax to ensure proper impregnation.

Paraffin wax I …… 2hours

Paraffin wax II …… 2hours

Embedding: Impregnated tissues were placed in molds (tissue cassette) with their labels and then fresh melted wax was poured in it and allowed to settle and solidify. Afterwards they were immersed in cold water to cool it rapidly. After the blocks were completely cooled, they were cut into individual blocks and each trimmed.

Staining of Processed Tissues Principle: Hematoxylin is a basic dye and thus has affinity for the acidic part of the cellular component which is the nucleus. Therefore, the nucleus stains blue while eosin on the other hand is an acidic dye thus has affinity for the basic component of the cells which is the cytoplasm therefore it stains it pink which is the color of the dye. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

APPENDIX III

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

1. The section was dewaxed in two changes of xylene for 2minutes each.
2. The section were taken through descending grades of alcohol. From absolute alcohol for 2minutes to 90% alcohol for 1minutes, 70% alcohol for 1minutes
3. The slides were washed in running tap water for one minutes.
4. Tissue sections were stained in hematoxylin for 10minutes
5. The sections was rinsed in distilled water for 30 seconds.
6. The sections was then differentiated in 1% acid alcohol for 15seconds
7. After that, the sections were rinsed in distilled water for 5minutes.
8. The sections was counterstained with 1% eosin for 5minutes
9. The sections was washed in running tap water for 30seconds
10. Sections was dehydrated by passing through ascending grades of alcohol (70%, 90%, and 100%) for 1minutes each.
11. The section was cleared in two changes of xylene for 2minutes each
12. The section was mounted with DPX and viewed microscopically using the objectives lens.

APPENDIX IV

ETHICAL APPROVAL CERTIFICATE

APPENDIX V

PLANT VERIFICATION CERTIFICATE

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Jatropha curcas* Linn.

Family: Euphorbiaceae

Common Name: Physic nut, Purging nut, Poison nut, Bubble bush, Barbados nut

Voucher Number: UBH-J404g

Student Name: Imobekhai Raphael

Plant Identification and Voucher Number Issued by:

23/05/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MECOSON, LMBOSON, MAEIAN; MFBAN Nigeria).

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
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Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Tetrapleura tetraptera* (Schumach & Thonn.) Taub.

Family: Fabaceae

Common Name: Soup Perfume, "Perekese"

Voucher Number: UBH-T472d

Student Name: Imobekhai Raphael

Plant Identification and Voucher Number Issued by:

23/05/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MECOSON, LMBOSON, MAEIAN; MFBAN Nigeria).