EVALUATION OF ACUTE AND SUB ACUTE TOXICITY OF FUMARATE

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACOLOY AND TOXICOLOGY, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF PHARM. D DEGREE

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CERTIFICATION

This is to certify that this project was carried out by Odore Samson in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State

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DEDICATION

This work is dedicated first to God almighty, my strength and my motivator. I also dedicate the success of this work to my big Brother, Engr Felix Odore and to my parents, Mr and Mrs John Odore for their continuous support and motivation.

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ABSTRACT

Background: Fumarate is an organic salt derived from fumaric acid, a dicarboxylic acid found in many fruits and vegetables. It plays a role in the citric acid cycle, which is a series of chemical reactions that occur in cells to produce energy. Fumarate is also used as a food additive and medication for treating certain medical conditions such as iron-deficiency anemia, psoriasis, and multiple sclerosis.

Aim: The aim of the study was to evaluate the effect of acute and subacute administration of fumarate.

Methods: The experiment involved the use of Wistar rats as the test animals. For the 28-day exposure to fumarate, the rats' body weight, blood, and organs were monitored and collected. A separate acute toxicity study was conducted in mice to determine the LD50 of fumarate. Afterward, a sub-acute toxicity study was performed on rats, in which the animals were given different doses of fumarate for a period of time and their body weight, blood, and organs were monitored and collected. The data collected was analyzed statistically using one-way ANOVA followed by Tukey's test.

Results: The LD50 in the acute toxicity study in mice was found to be 3,807.89 mg/kg. The body weight of rats exposed to Fumarate did not significantly change throughout the study. Fumarate administration led to significant changes in some white cell parameters and did not significantly affect the red blood cell indices. Normal levels of total cholesterol and triglycerides was observed as well as albumin and total protein levels in both male and female rats. Histological examination of the different organs revealed Fumarate to be hepatotoxic.

Conclusion: Fumarate possess a wide margin of safety in mice whereas subacute furmarate exposure in rats had significant effects on haematological parameters, and white blood cels, suggesting potential health risk, the aoita, heart and kidney tissues were normal. However, care must be taken due to it effects on the liver.

Keywords: Fumarate, Acute toxicity, Subscute toxicity, Hematological parameters

Histopathological examination.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Fumarate is a white crystalline powder that is commonly used in pharmaceuticals, food additives, and animal feed. It is the conjugate base of fumaric acid, a dicarboxylic acid, and has a chemical formula of C4H4O4. Fumarate plays a significant role in cellular metabolism, particularly in the citric acid cycle, also known as the Krebs cycle. It is the salt of fumaric acid, which is a dicarboxylic acid found in various natural sources such as fruits, vegetables, and wine.

In the human body, fumarate is an intermediate in the citric acid cycle, where it participates in the conversion of succinate to malate. This process is crucial for generating energy through aerobic respiration. Furthermore, fumarate serves as an electron acceptor during cellular respiration, forming succinate through the enzyme succinate dehydrogenase.

1.2 Fumarate and citric acid cycle

Citric acid cycle, also known as the TCA cycle or Krebs cycle, is a significant metabolic pathway in all living beings. It is vital in generating energy for cells by oxidizing acetyl CoA to produce ATP, which is the primary source of energy for cells. The cycle comprises a sequence of enzymatic reactions that transpire in the mitochondria, a cellular organelle responsible for energy production.

The TCA cycle was discovered by Sir Hans Adolf Krebs in 1937, and he was awarded the Nobel Prize in Physiology in 1953 for his research on the subject. Since then, the cycle has been meticulously studied, and its role in cellular metabolism has been firmly established.

The TCA cycle initiates with citrate's formation from the amalgamation of acetyl CoA and oxaloacetate, triggered by the enzyme citrate synthase. The enzyme aconitase converts citrate into isocitrate which is then oxidized to α-ketoglutarate by the enzyme isocitrate dehydrogenase. Further, α-ketoglutarate is transformed to succinyl CoA by the enzyme α-ketoglutarate dehydrogenase complex. Succinyl CoA is eventually converted to succinate by the enzyme succinyl CoA synthetase. Fumarate is formed from succinate by the enzyme succinate dehydrogenase, which is embedded in the inner mitochondrial membrane and is part of the electron transport chain. Fumarate is then transformed to malate by the enzyme fumarase, and malate is reversed back to oxaloacetate by malate dehydrogenase, thus completing the cycle.

The TCA cycle's role in energy metabolism is critical as this process aids the production of ATP via oxidative phosphorylation, which occurs in the mitochondria. Additionally, the cycle generates reducing equivalents in the form of NADH and FADH2, which are used by the electron transport chain to produce ATP. Moreover, this cycle provides precursors for the biosynthesis of amino acids, nucleotides, and lipids.

Altogether, the TCA cycle is an essential metabolic pathway that plays a central role in energy metabolism. It involves a series of enzymatic reactions that occur in the mitochondria and is responsible for producing ATP, reducing equivalents, and precursors for biosynthesis. The TCA cycle has been studied extensively, and its role in cellular metabolism has been well established.

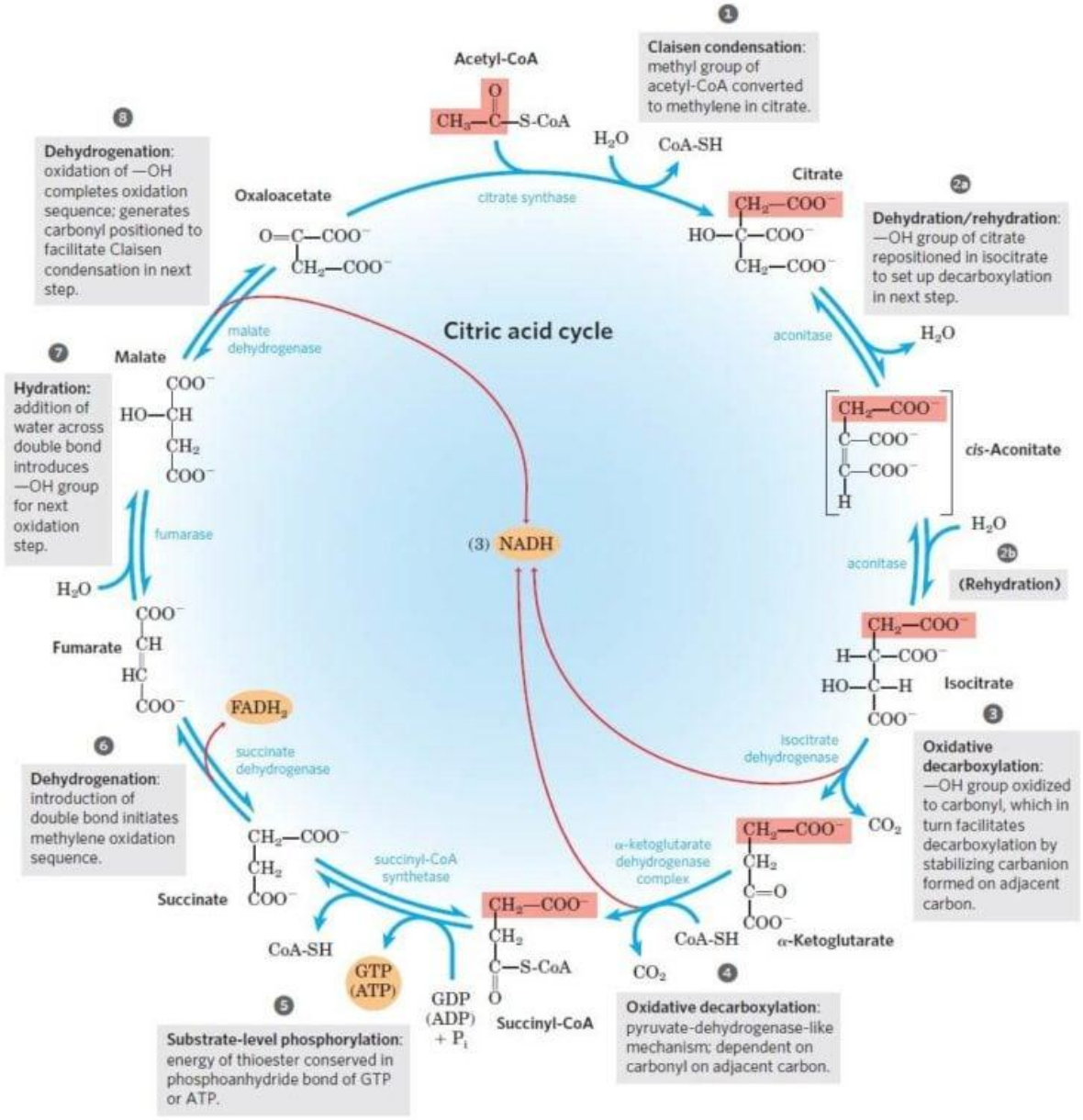


Fig 1: Reactions of the citric acid cycle

1.3 Sources of fumarate

Fumarate is a compound found in a variety of natural sources, including food, plants, and the human body. Food sources that are high in fumarate include fruits like watermelon, apples, and grapes, as well as vegetables like spinach, broccoli, and carrots. Legumes such as lentils and beans are also rich in fumarate.

Fumarate is sourced from various pharmaceutical products, including fumaric acid esters. One example is dimethyl fumarate, which is used to treat psoriasis. This compound is believed to work by reducing inflammation and preventing the formation of free radicals, which can damage cells. The citric acid cycle is an important metabolic pathway that produces energy for the body. Fumarate is a key component of this cycle, and its synthesis and metabolism play a vital role in the production of cellular energy.

1.4 Fumarate and pathological conditions

Fumarate, a derivative of fumaric acid, is an essential component in various physiological processes in the human body (Nguyen et al., 2007). However, elevated levels of fumarate can lead to pathological conditions, including psoriasis, cancer, and mitochondrial disorders due to its ability to inhibit enzymatic activity in the citric acid cycle, disrupting cellular energy production and metabolism (Tillmann & Ernst, 2018). Despite this, certain derivatives of fumarate, such as fumaric acid esters (FAEs), have shown promise in treating specific conditions, such as psoriasis and multiple sclerosis, by modulating the immune response, reducing inflammation, and promoting the growth of skin cells (Holmes, 2017).

Furthermore, prior research has shown that fumarate has antioxidative properties, making it an effective drug for treating inflammatory diseases like rheumatoid arthritis and Crohn's disease (Rogers, 2019). In addition, recent studies have also shown the role of fumarate in treatment of salt-sensitive hypertension (Kopkan & Červenka, 2019). Fumarate is commonly used as a food additive due to its ability to regulate pH levels in food and enhance flavor and texture (de Oliveira Rios et al., 2017).

On the other hand, research suggests that fumarate may play a role in cancer development. Mutations in fumarate hydratase have been linked to hereditary leiomyomatosis and renal cell carcinoma (HLRCC), which can lead to tumor growth.

While fumarate is generally regarded as safe for consumption, further comprehensive research is warranted to explore its potential adverse effects on human health (Franssen et al., 2011). Existing literature primarily focuses on its role in metabolism, energy production, and other biochemical pathways, with limited studies on its toxicological effects and toxicokinetic properties. Thus, it is crucial to carefully consider the negative aspects of using fumarate derivatives before implementing it into clinical practice or other applications.

1.5 Fumarate and cardiorenal system

Fumarate's role in coordinating biological systems is multifaceted and encompasses various cellular processes. It serves as a signaling molecule that directly interacts with proteins involved in gene expression, cell differentiation, and apoptosis, modulating their activity and function.(West et al., 2017)

One notable example of fumarate's coordination of biological systems is its interaction with the hypoxia-inducible factor (HIF) pathway. HIF is a transcription factor responsible for regulating genes involved in oxygen homeostasis and angiogenesis. By inhibiting the activity of an enzyme called prolyl hydroxylase domain-containing protein 2 (PHD2), fumarate prevents the degradation of HIF under normoxic conditions. This stabilization of HIF leads to increased expression of its target genes, enabling cells to adapt to low oxygen levels.

Fumarate's involvement in coordination extends to the Nrf2 pathway, which regulates antioxidant response genes. Fumarate activates Nrf2 by modifying a cysteine residue on its

regulatory protein Keap1. This modification results in increased expression of antioxidant enzymes, offering protection against oxidative stress (Helps et al. 2017)

The ability of fumarate to coordinate biological systems through direct interactions with key proteins underscores its significance in maintaining cellular homeostasis and adapting to changing environmental conditions. Further research into these mechanisms holds promise for developing new therapeutic strategies to address diseases associated with disrupted cellular signaling pathways.

In addition to its role in cellular coordination, fumarate plays a significant role in regulating blood pressure. It interacts with various pathways in the human body, particularly the renin-angiotensin-aldosterone system (RAAS), which controls vasoconstriction and vasodilation, crucial factors in blood pressure regulation.

Moreover, fumarate inhibits angiotensin-converting enzyme (ACE), a target of many blood pressure medications. This inhibition aids in lowering blood pressure and promoting healthy blood flow.

Furthermore, studies have demonstrated that fumarate promotes vasodilation by activating potassium channels in smooth muscle cells, leading to improved blood flow and ultimately contributing to lower blood pressure levels. Additionally, research suggests that fumarate may be involved in regulating the production and metabolism of nitric oxide (NO), which promotes vasodilation while maintaining healthy blood flow.

The findings on fumarate's impact on blood pressure regulation and cardiovascular health suggest its potential as a therapeutic target for hypertension and related conditions. However, further research is necessary to fully comprehend its effects on blood pressure regulation.

Overall, the interactions between fumarate and the RAAS, along with ACE inhibition, promote vasodilation. Additionally, fumarate's potential involvement in NO production makes it an intriguing compound worthy of further exploration as a potential treatment for hypertension and other cardiovascular conditions. Future research could investigate the specific effects of fumarate on potassium channels in promoting vasodilation while examining its interactions with other pathways involved in blood pressure regulation.

Furthermore, exploring optimal dosages can help achieve therapeutic benefits while minimizing potential side effects. In conclusion, continued investigation into the mechanisms of action and clinical applications of fumarate may uncover new treatments for hypertension, stroke, cancer, and other diseases.

Recent studies have indicated that fumarate may also possess antioxidant properties that benefit cardiovascular health. Oxidative stress, which arises from an imbalance between reactive oxygen species production and antioxidant defenses, plays a role in the pathogenesis of hypertension and atherosclerosis. Fumarate's ability to scavenge free radicals and enhance endogenous antioxidants like glutathione helps reduce oxidative damage to blood vessels, thereby improving endothelial function.

Additionally, fumarate has been investigated for its neuroprotective effects using ischemic stroke models. Pre-treatment with fumarate has been found to reduce the size of infarction.

1.6 Toxicity testing

Studying both acute and chronic toxicity of fumarate is of utmost importance in order to comprehensively understand its potential adverse effects on human health.

Acute Toxicity Testing

Acute toxicity refers to the immediate harmful effects that occur shortly after exposure to a substance, while chronic toxicity refers to the long-term or delayed effects that may develop over time.

Investigating acute toxicity is crucial for assessing the immediate risks associated with fumarate exposure. This includes determining its LD50 (lethal dose 50), which represents the dose at which 50% of exposed organisms experience lethal effects. It is done for a period of 24 hours. Understanding acute toxicity can help establish safe exposure limits and guide emergency response protocols in cases of accidental or intentional ingestion or inhalation of fumarate.

Chronic Toxicity Testing

Studying chronic toxicity is essential for assessing the potential long-term health risks associated with continuous or repeated exposure to fumarate. Chronic toxicity studies involve evaluating the effects of prolonged exposure on various organs, systems, and physiological processes. The chronic toxicity study was conducted for 28days, during which the drug was orally administered, and after 28days toxicity parameters such as body weight changes, relative organ weight, hematology and organ histopathology were examined. In order to ensure public health and safety, it is important to conduct comprehensive research on both acute and chronic toxicity of fumarate using appropriate experimental models and standardized methods.

Investigating the acute and chronic toxicity of fumarate is of paramount importance due to various factors. Foremost, it is crucial for human health and safety as comprehending the acute and chronic toxicity of fumarate can guarantee the safety of individuals who may interact with it. Short-term studies offer insight into the immediate impact of high doses of fumarate on various

organ systems, while long-term studies can help evaluate the long-term health implications of continuous contact with the substance.

Secondly, regulatory agencies across the globe require toxicity data on substances such as fumarate to ensure their safe use. Such agencies establish guidelines for acceptable concentrations of substances in various products and exposure limits. Examining the toxicity of fumarate is necessary to meet these regulatory requirements and guarantee compliance.

Thirdly, fumarate is a common ingredient in a range of products such as pharmaceuticals, food additives, and industrial chemicals. Understanding the toxicity of fumarate is crucial in the development and formulation of these products and ensures their safety for human use. This knowledge can also guide the development of safer alternatives when necessary.

Fourthly, the release of fumarate into the environment can have adverse effects on plants, aquatic organisms, and other non-target organisms. Researching its toxicity helps evaluate the potential harm it may cause to these organisms and guide environmental risk assessments. This knowledge also supports in developing appropriate mitigation measures to safeguard ecosystems.

Lastly, investigating the acute and chronic toxicity of fumarate supports in the overall risk assessment and management of the substance. The data obtained from such studies help in identifying the dose-response relationship, target organs or systems affected, and potential for cumulative effects, which are essential in evaluating the risk associated with the use of fumarate and developing strategies to mitigate or eliminate exposure and adverse effects.

In summary, exploring the acute and chronic toxicity of fumarate is indispensable in ensuring human health and safety, regulatory compliance, product development, assessment of environmental impact, and effective risk assessment and management.

1.7 Rationale of the study

Fumarate is an essential component in various physiological processes. However, recent concerns have arisen regarding its potential toxicological effects. Despite it beneficial traits, the toxicological aspects of fumarate have not received the same level of attention in scientific literature. While fumarate is generally regarded as safe for consumption, further comprehensive research is warranted to explore its potential adverse effects on human health. To date, limited studies have focused specifically on the toxicological effects of fumarate. Existing literature primarily explores its role in metabolism, energy production, and other biochemical pathways. While some studies indirectly touch upon potential adverse effects, they fail to provide a comprehensive analysis or establish clear cause-effect relationships.

For instance, the knowledge of its toxicokinetic properties remains limited (Tillmann & Ernst, 2018). The scarcity of research on fumarate's toxicity can be attributed to several factors. Firstly, fumarate has long been considered safe due to its natural occurrence in the human body and involvement in vital biological processes. Consequently, it has received less attention compared to other potentially harmful substances.

Moreover, funding priorities often favor investigations into more widely recognized toxins or substances with established adverse effects. This bias limits resources available for studying lesser-known compounds like fumarate.

Additionally, the complex nature of fumarate's metabolism and its involvement in various biochemical pathways make it challenging to study its toxicological effects. Researchers need to consider multiple factors, such as dose-response relationships, potential interactions with other compounds, and the specific mechanisms through which fumarate may exert toxicity.

This research is intend on evaluating excessive fumarate intake if it can lead to a variety of health issues, including kidney dysfunction, liver damage, and cardiovascular problems.

Despite the growing interest in fumarate toxicity, there are some limitations in our understanding of its exact mechanisms and effects. One limitation is the lack of comprehensive human studies that directly assess the toxic effects of fumarate. Most of the existing research has been conducted on animal models or in vitro studies, which may not fully represent the complexities of human physiology and metabolism.

Fumarate toxicity research often focuses on high-dose exposures rather than chronic low-level exposures that may be more relevant to real-life scenarios. The long-term effects of low-level fumarate consumption and its potential to accumulate in the body over time are still not well understood. This is as well the focus of this study.

Despite these challenges, it is crucial to conduct further research on the toxicological effects of fumarate. Understanding its potential adverse effects is essential for ensuring public health and safety. Additionally, comprehensive knowledge of fumarate's toxicity can help inform regulatory decisions regarding its use in pharmaceuticals, food additives, and other applications.

1.8 Aim and objectives

The aim of the study was to evaluate the acute and subacute toxicity effects of Fumarate.

The objectives are;

1) To determine the acute toxicity of fumarate using lorke's model to demonstrate the immediate risks of Fumarate exposure.
2) To evaluate sub acute toxicity studies inorder to access the long-term effects of Fumarate.

CHAPTER TWO

MATERIALS AND METHOD

2.1 MATERIALS

DRUGS AND CHEMICALS

Fumarate (Sigma-Aldrich), Distilled water (EMD Millipore), Formaldehyde (Fisher Scientific), Chloroform (Thermo Fisher), Normal saline (Baxter)

APPARATUS

Syringes and needles, orogastric tube, beakers, measuring cylinders, sample bottle, electronic weighing balance, filter paper

2.2 EXPERIMENTAL ANIMALS

Male and female albino rats weighing between 100 – 150 g, and albino mice weighing between 20 to 30g, bred in the Department of Pharmacology, University of Benin, Benin City, Nigeria were used for the study. The rats were housed under standard laboratory conditions of light/dark cycle for 12 h at temperature 27 ± 2 °C with free access to standard commercial chow and water ad-libitum. The rats and mice were allowed to acclimatize for two weeks before the experiment commenced.

2.3 ACUTE TOXICITY STUDIES IN MICE

The single-dose acute oral toxicity study was evaluated following the Lorkes Method. This method has two phases which are phases 1 and 2 respectively.

Phase 1

This phase requires twelve mice. The twelve animals were divided into three groups of four animals each. Each group of animals (n=12) are administered different doses (10, 100 and 1000 mg/kg) of fumarate. The animals were placed under observation for 24 hours to monitor their behaviour as well as if mortality will occur.

Phase 2

This phase involves the use of three mice (n=3), which are distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of fumarate and then observed for 24 hours for visual observations of mortality, various changes in physical appearance, behaviour (salivation, lethargy), and any injury or illness.

Then the LD 50 was calculated by the formula:

LD 50=√D0 X D100

D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality.

2.4 SUBACUTE TOXICITY STUDY IN RATS

2.4.1 TREATMENTS

Thirty 30 (male and female) rats of average weight 130 ± 20 g were randomly divided into 4 groups of 8 rats each. Group 1 rats containing 4 male and 4 female served as the control and were administered distilled water (1ml/kg), once daily, orally for 28 days. Group 2–4 animals were administered orally 50, 150 and 500 mg/kg dose of fumarate respectively once daily for 28 days. The animals were observed daily for general health and clinical signs of toxicity, whereas body weight changes were recorded on days 0, 7, 14, 21, and 28 of the experiment.

2.4.2 SAMPLE COLLECTION

All animals were sacrificed by cervical dislocation 24 h after the last exposure to fumarate. After twenty-eight (28) days of treatment, the animals was sacrificed under chloroform anaesthesia. Blood samples were collected from the abdominal aorta under anesthesia with ether in two types of tubes: one with EDTA and the other without additives. The anticoagulated blood (tube with EDTA) was analyzed immediately for hematological parameters. The second tube was centrifuged at 4000 rpm at 4°C for 10 min to obtain the hematological parameters. Additionally, liver, kidney, heart and aiota were dissected and weighed on analytical balance, and kept in bottle containing 10% formalin for histopathological studies.

2.4.3 WEEKLY BODY WEIGHT

The body weight of each rat was carefully monitored before study commencement, once weekly during the study.

2.4.4 MORTALITY AND TOXICITY SIGNS

The visual observations of mortality, various changes in physical appearance, behaviour (sleepy, salivation, lethargy), and any injury or illness were conducted once daily for 28 days.

2.4.5 HAEMATOLOGICAL PARAMETERS

After collecting blood from cardiac puncture into EDTA containing tubes, various parameters were evaluated at Hematological Lab, University of Benin Teaching hospital, Benin city. The haematological parameters, like the blood samples filled into the EDTA bottles will be analysed to determine the red blood cell (RBC), haematocrit (HCT), haemoglobin concentration (Hb), platelet count (PLT), Lymphocytes (LY%), total white blood cell (WBC) counts and its

differentials, mean corpuscular volume (MCV), mean corpusular haemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were estimated.

2.4.6. BIOCHEMICAL ESTIMATIONS

Blood collected in non-heparinized tubes were then centrifuged at 4000 r/min for 10 min. The serum separated was analysed at Pathology Lab, University of Benin Teaching hospital , Benin city, for various parameters such as sodium, potassium, chloride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), creatinine (Cr), urea, Total bilirubin (TB), Globulin (GLO), total triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoproteins (LDL), and serum electrolytes.

2.4.7. HISTOPATHOLOGY STUDY

The organs, namely liver, heart, aorta and kidney were carefully excised and weighed. These organs were preserved in a fixation medium of 10% buffered formalin for histopathological study.

CHAPTER THREE

RESULTS

3.1. ACUTE TOXICITY ASSESSMENT.

Fumarate at a dose of 10 mg/kg, 100mg/kg and 1000mg/kg produced no mortality for 24 hours. There were no signs of changes in the behaviour patterns, skin, eyes, salivation, and diarrhoea of the mice.

Fumarate orally administered at a dose of 1600 mg/kg and 2900 mg/kg to mice did not induce any death or toxic symptoms in treated mice. Animals displayed normal behavior throughout the study and survived until the end of the 24-hour experiment period. During the entire observation period, they did not present any significant clinical alteration. Whereas, Fumarate administered at a dose of 5000 mg/kg to produce anxiety, delirium and mortality within an hour of administration.

LD 50 ESTIMATION

The median lethal dose (LD50) is defined as the dosage that kills 50% of the mice exposed.

LD 50 is calculated by the formula:

LD 50=$\sqrt{D0\ X\ D100}$

Where; D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality.

D0=2900, D100=5000

LD 50=$\sqrt{2900\ X5000}$

LD 50 = 3 807.89 mg/kg

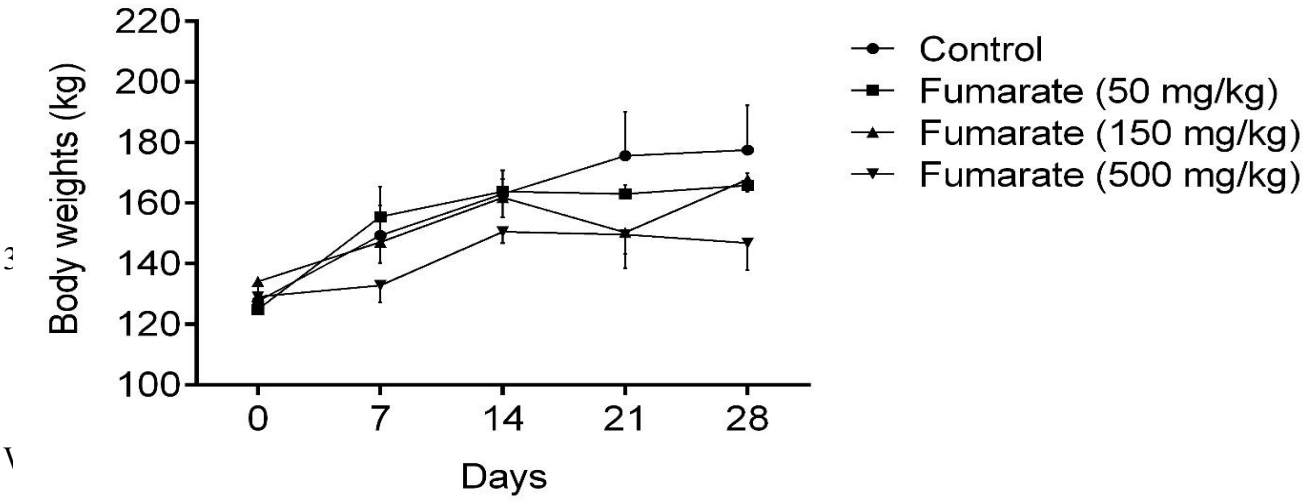


Fig 1: Weekly body weights of male rats treated with fumarate for 28 days.

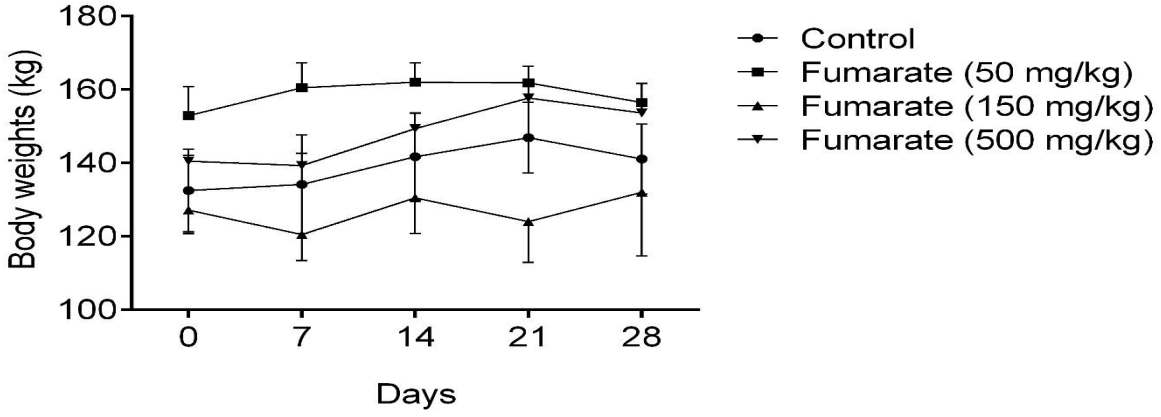


Fig 2: Weekly body weights in female rats treated with fumarate for 28-days. Control (distilled water)

Haematological parameters

Table 1: effect of 28-day administration of fumarate in white cell parameters.

Sex	Parameter	Fumarate (mg/kg			
		Control	50	150	500
Male	WBC (x10^3/µL)	6.8±0.3	18.1±1.8**	11.5±1.8	12.9±0.9**
	LYM (x10^3/µL)	5.9±0.1	14.0±1.5*	9.9±1.7	15.3±1.5*
	MON (x10^3/µL)	1.1±0.1	2.7±0.4*	1.2±0.1	2.7±0.6*
	GRA (x10^3/µL)	0.3±0.0	1.4±0.5	0.50±0.1	1.1±0.3
	LYM%	81.3±0.7	77.8±4.0	84.6±1.6	80.7±2.5
	MON%	13.6±0.3	14.8±1.6	11.0±1.2	13.7±1.6
	GRA%	3.8±0.0	7.4±2.6	4.4±0.6	5.6±0.9
Female	WBC (x10^3/µL)	6.5±1.5	12.8±1.3*	16.2±1.1**	12.9±0.9*
	LYM (x10^3/µL)	5.1±1.2	10.7±1.0*	13.5±0.6**	10.5±0.8*
	MON (x10^3/µL)	1.0±0.2	1.6±0.3	1.8±0.3	1.7±0.2
	GRA (x10^3/µL)	0.2±0.0	0.6±0.1	0.9±0.3*	0.7±0.1
	LYM%	79.7±0.4	83.6±1.6	84.0±2.0	81.2±2.3
	MON%	16.0±0.5	11.8±1.1	10.7±0.9	13.3±1.6
	GRA%	3.7±0.9	4.6±0.5	5.3±1.1	4.3±0.7

**$p<0.01$, *$p<0.05$ compared to control (distilled water, 3 mL/kg po) . WBC= white blood cells; PLT = platelets; MPV = mean platelet volume; GR = granulocytes count; MO = monocytes/ eosinophils; LY = lymphocytes.

Table 2: Some haematological parameters in fumarate-treated rats after 28-days.

Sex	Parameter	Fumarate (mg/kg)			
		Control	50	150	500
Male	RBC ($x10^6/\mu L$)	5.7±0.1	6.8±0.1	5.1±0.9	5.8±0.1
	HGB (g/dL)	11.9±0.5	14.2±0.8	10.9±0.7	13.2±0.6
	HCT (%)	35.1±0.2	39.5±3.7	29.4±5.6	35.9±1.5
	MCV (μm^3)	61.7±0.9	58.2±4.6	57.2±1.2	62.4±1.7
	MCH (pg)	23.3±0.7	31.9±10.4	22.2±0.6	22.8±0.6
	MCHC (g/dL)	36.0±0.5	36.3±1.1	36.9±0.4	36.6±0.1
	RDWC (%)	17.3±0.3	17.4±0.2	19.1±0.8	18.5±1.3
	RDWS (μm^3)	38.7±0.2	36.0±3.0	39.6±1.6	41.7±1.1
Female	RBC ($x10^6/\mu L$)	6.8±0.2	6.5±0.4	6.2±0.3	6.3±0.3
	HGB (g/dL)	13.4±0.7	14.4±0.8	13.7±0.7	14.1±0.6
	HCT (%)	36.2±1.7	37.3±2.2	35.2±1.7	36.0±1.2
	MCV (μm^3)	53.0±3.8	57.8±0.6	56.8±0.6	56.9±1.1
	MCH (pg)	19.7±1.6	22.4±0.3*	22.2±0.6*	22.3±0.3*
	MCHC (g/dL)	37.1±0.3	38.9±0.3	39.0±0.6*	39.2±0.2*
	RDWC (%)	17.0±0.5	15.8±0.8	16.7±0.6	16.3±0.1
	RDWS (μm^3)	33.9±2	34.5±1.5	34.8±0.6	34.8±0.6

*$p<0.05$ vs control (distilled water, 3 mL/kg,po). Hb = Hemoglobin; HCT = Hematocrit; RBC = red blood cells; PLT = platelets; MPV = mean platelet volume; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; MCH = mean corpuscular hemoglobin; RDWc = red cell distribution width; PDW = platelet distribution width.

ex	Parameter		Fumarate (mg/kg		
		Control	50	150	500
Male	ALP (U/L)	264±7.4	385±57.3	419±28.9	343.0±68
	ALT (U/L)	74.3±13.5	98.3±6.8	119.0±9.5*	130.5±14.5*
	ABT (U/L)	319.7±9.8	298.2±22.5	376.0±11.8	358.0±14.0
	TB (g/dL)	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.1
	CB (g/dL)	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	TP (g/dL)	6.9±0.0	6.7±0.2	6.7±0.2	6.7±0.1
	ALB (g/dL)	4.2±0.1	3.7±0.1*	3.7±0.1	3.9±0.2
	GLO (g/dL)	2.8±0.1	2.9±0.2	3.0±0.3	2.9±0.3
Female	ALP (U/L)	277.0±51.2	385.0±60.4	347.0±29.5	350.6±19.5
	ALT (U/L)	88.0±6.9	136.0±63.5	119±28.5	120.4±10.7
	ABT (U/L)	346.0±47.5	232.0±17.9	353.7±48.14	350.0±30.6
	TB (g/dL)	0.2±0.0	0.4±0.0	0.2±0.0	0.3±0.0
	CB (g/dL)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
	TP (g/dL)	7.0±0.2	6.8±0.4	6.9±0.3	6.7±0.3
	ALB (g/dL)	3.9±0.1	3.9±0.2	3.9±0.2	3.8±0.1
	GLO (g/dL)	3.1±0.1	2.9±0.3	3.1±0.3	3.0±0.4

Table 3: Liver function parameters in rats treated with fumarate for 28-days.

*$p<0.05$ compared to control. ALP, Alkaline Phosphatase; AST, Aspartate transaminase; ALT, Alanine transaminase; TB, Total Bilirubin; CB, conjugated Bilirubin; GLO, globulin. Control (distilled water, 3mL/kg, po).

Sex	Parameter	Fumarate (mg/kg			
	(mg/dL)	Control	50	150	500
	HDL	22.5±3.5	32±3.5	28.5±1.8	32±1
	LDL	10±0.0	18.3±2.6	17.0±2.0	16.0±0.0
Male	TCHOL	74.7±2.2	69.0±3.3	68.0±7.6	61.5±8.5
	HDL	27.5±0.6	28±2.7	24.3±1.6	28.3±0.6
Female	LDL	15.0±1.5	14.8±1.8	12.7±1.9	13.6±1.4
	TCHOL	60.3±2.9	61.5±3.3	68.0±7.6	65.0±3.7

Table 4: Cholesterol and lipoprotein levels in rats treated with fumarate for 28-days.

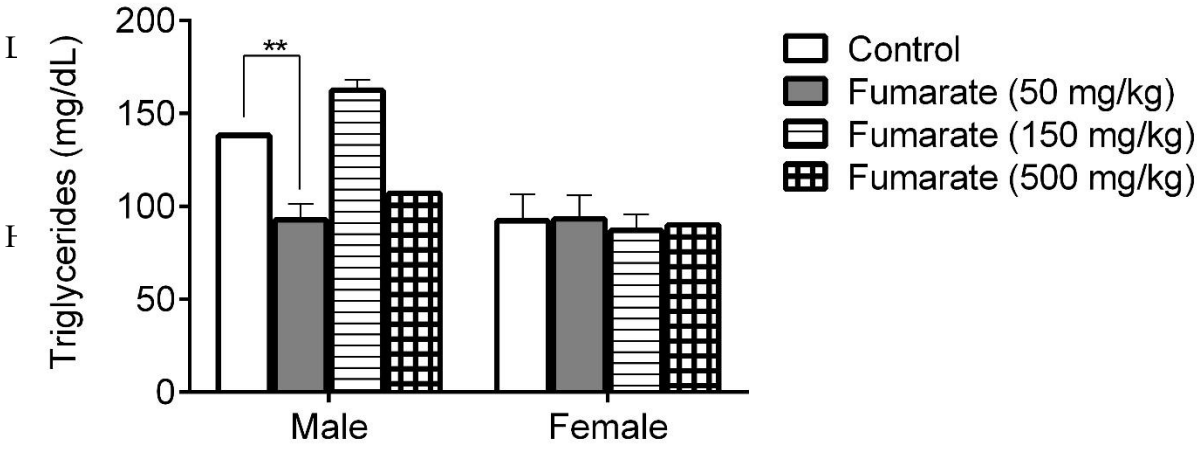
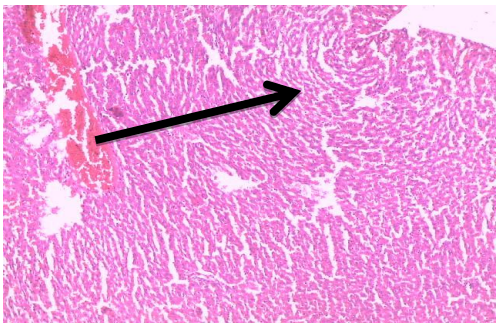


Fig 4: Triglycerides (TG) levels in rats treated with fumarate for 28 days.

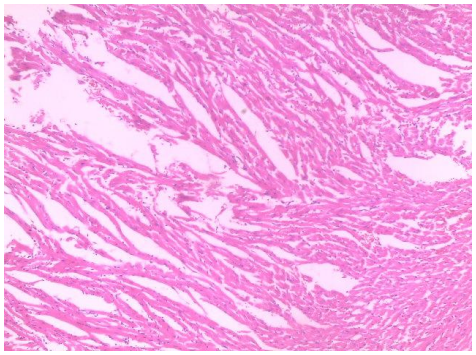
**p<0.01 compared to control (distilled water).

Histopathology examination

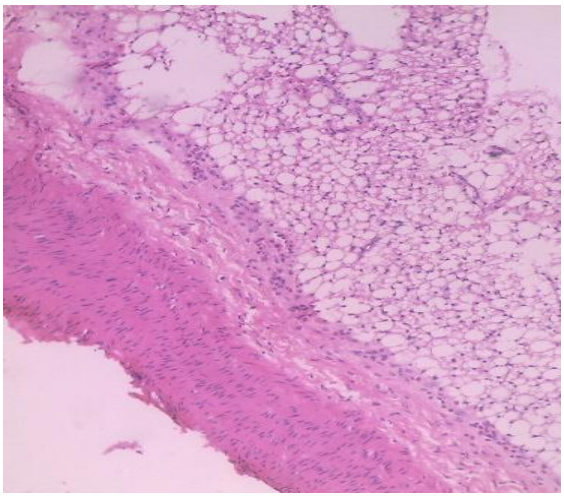
150MG/KG MALE

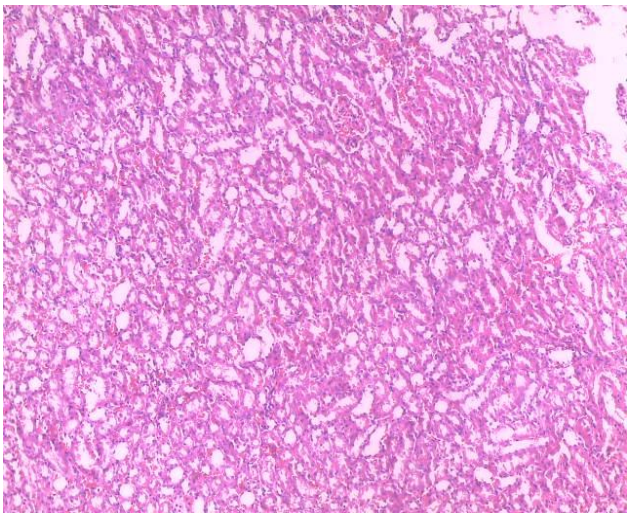


Liver: Section of the Liver Showing Severe Fatty Change (Steatosis)



Heart: Normal Heart

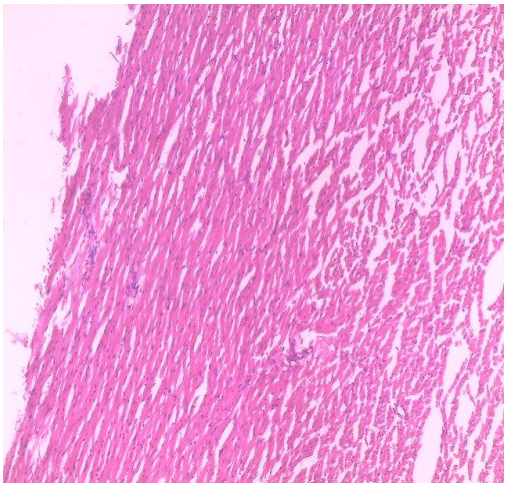




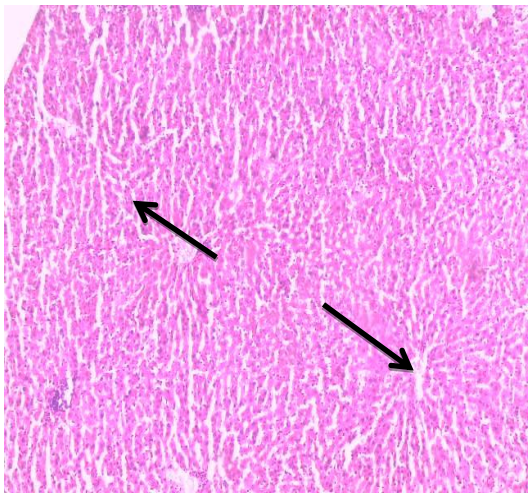
Aorta: Normal Aorta

Kidney: Normal Kidney

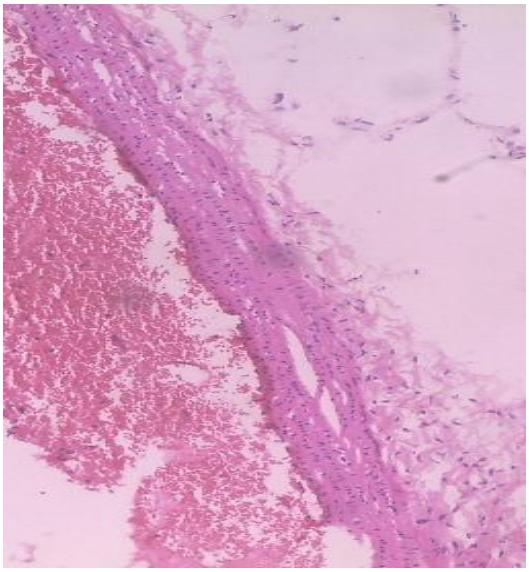
150MG/KG FEMALE



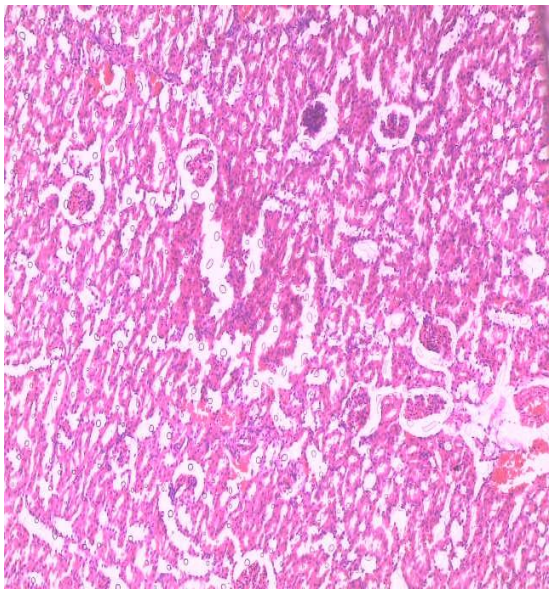
Heart: Normal Heart Liver:



Section of the Liver Showing Moderate Fatty Change (Steatosis)

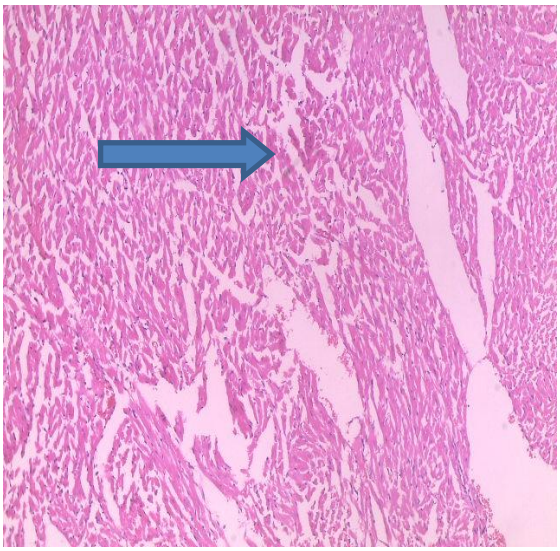


aorta: normal aorta

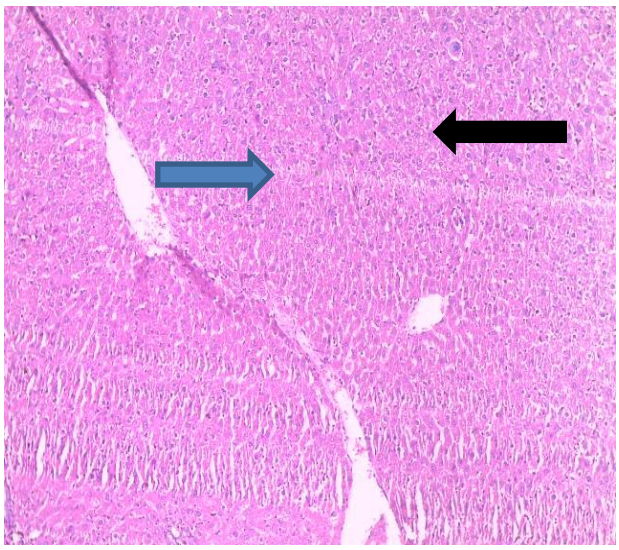


kidney: normal kidney

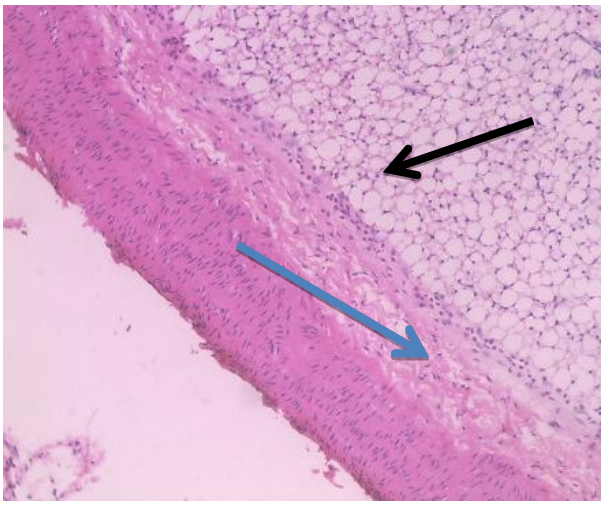
CONTROL MALE



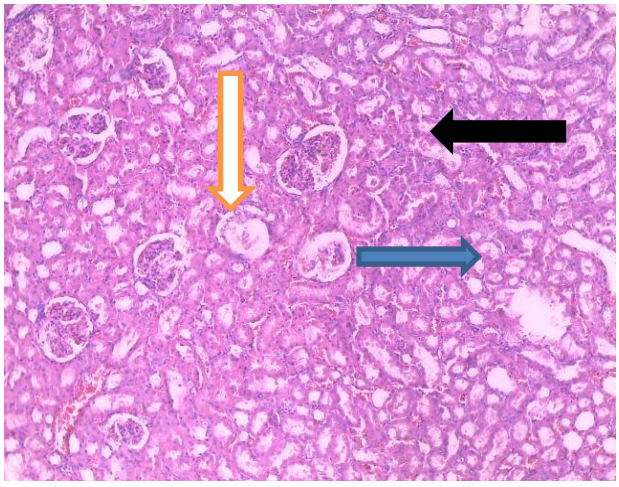
Heart: Normal Heart Muscle



Liver: Normal Liver Sections Showing Hepatocytes (Black Arrow) And Central Vein (Blue Arrow)



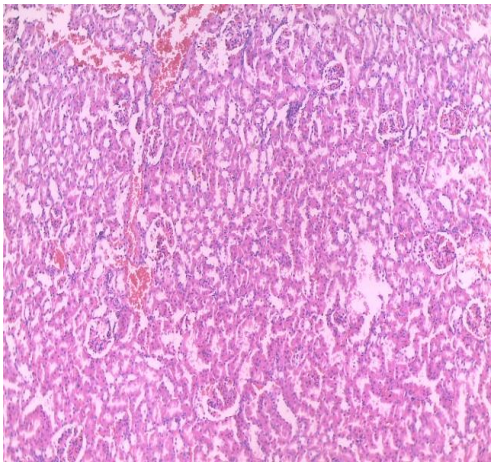
Aorta: Aorta: Normal Aortic Valve Consisting Of Endothelial



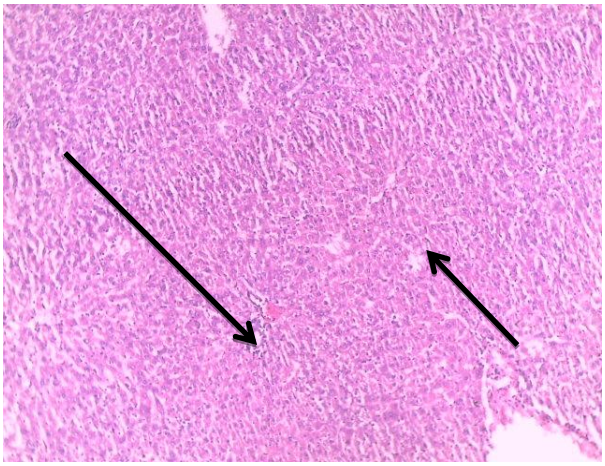
Kidney:Normal Kidney Sections

Aorta section: (Blue Arrow) Cells And Interstitial Cells(Black Arrow)Showing Glomerulus
Kidney section: (Black Arow), Bowman's Capsule(Blue Arrow) And Renal Tubules (White Arrow).

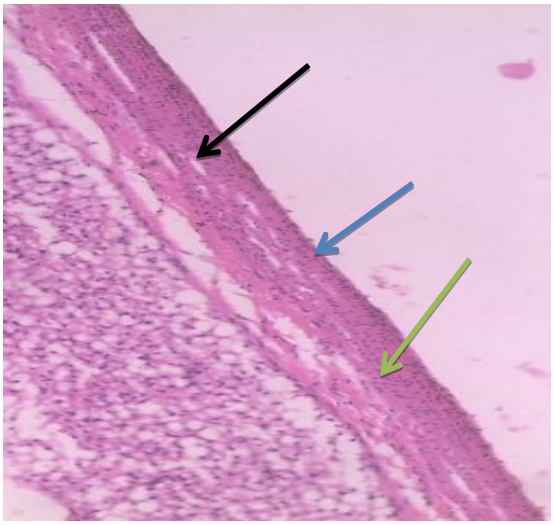
50MG/KG FEMALE



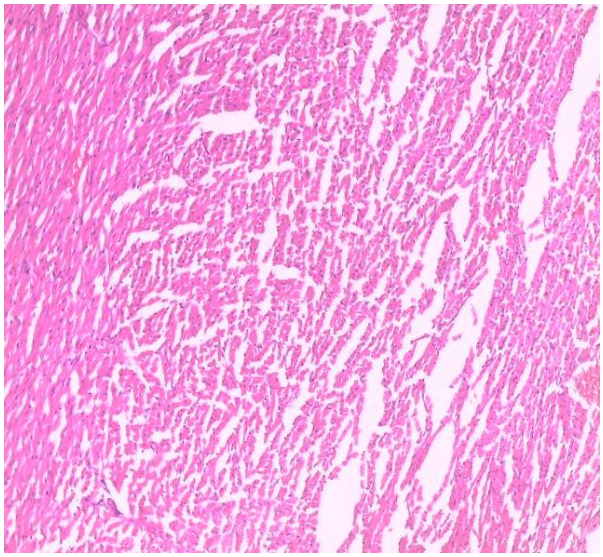
Kidney: Normal Kidney



Liver: Section of the Liver Showing Moderate Fatty Change (Steatosis)

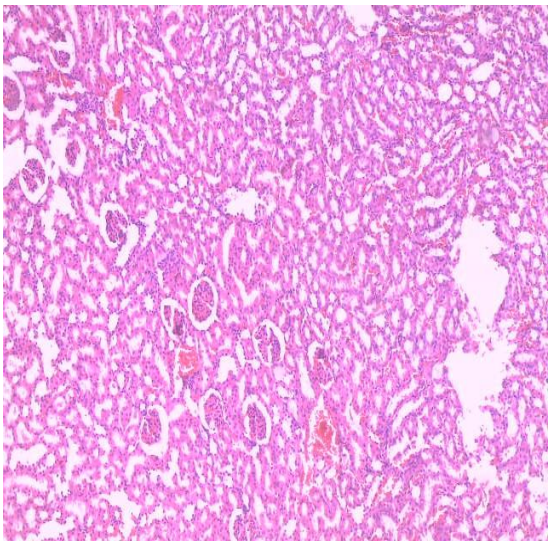


Aorta: Smooth Muscle Fibre (Black Arrow), Endothelial Cell Layer(Blue Arrow), Intestitium(Green Arrow)

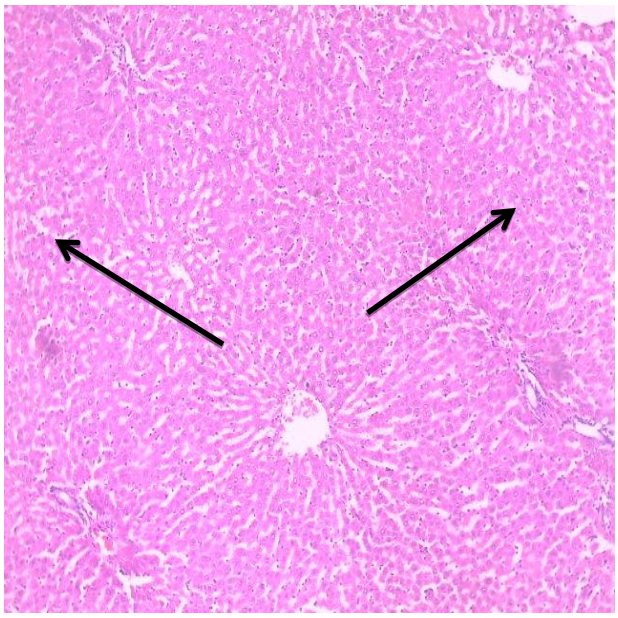


Heart: Normal Heart

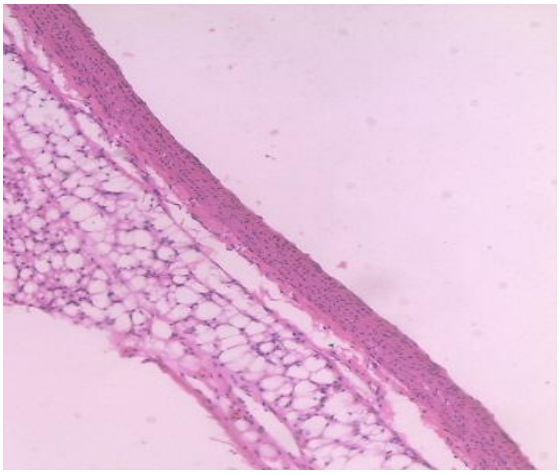
50MG/KG MALE



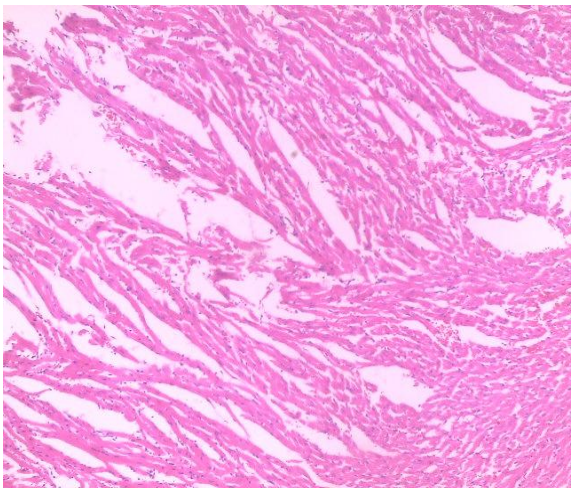
Kidney: Normal Kidney



Liver: Section of the Liver Showing Moderate Fatty Change (Steatosis)

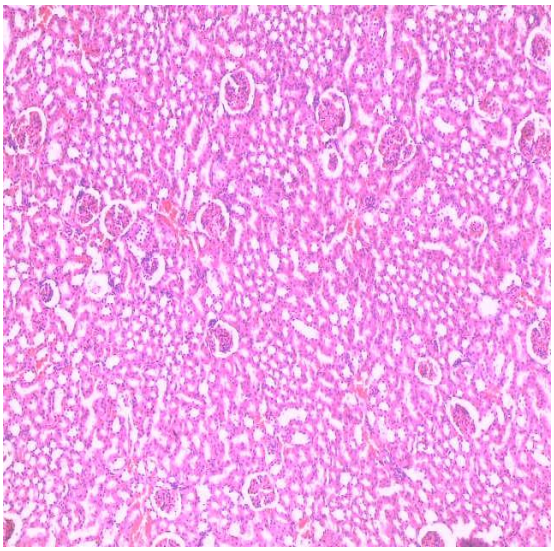


Aorta: Normal Aorta

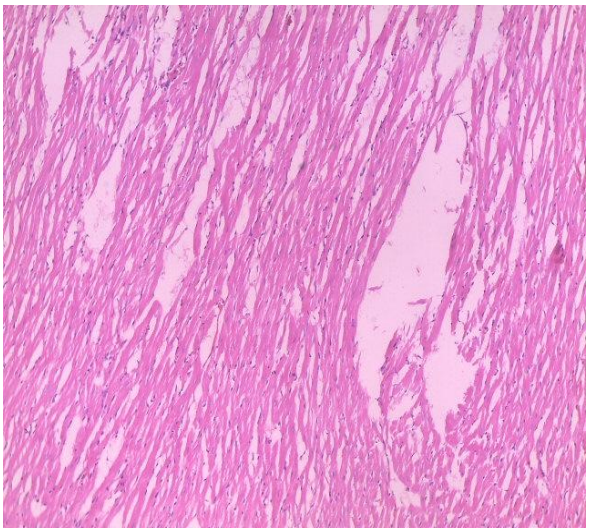


Heart: Normal Heart

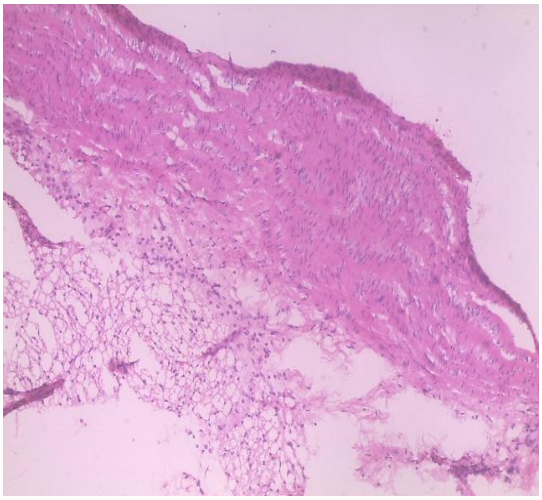
500MG/KG MALE



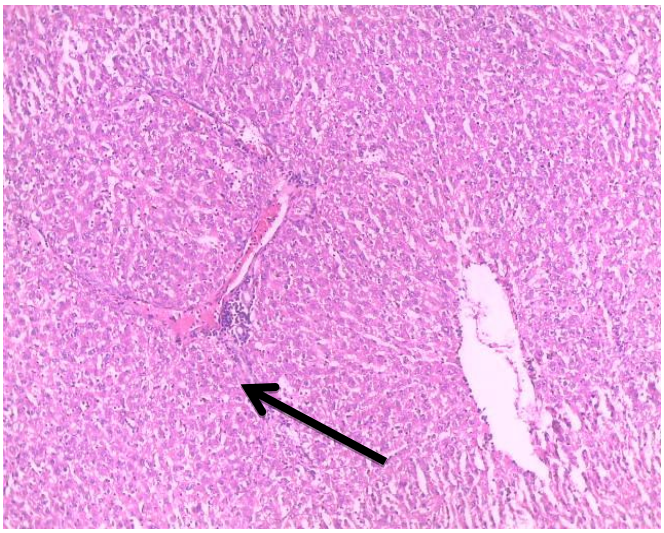
Kidney: Normal Kidney



Heart: Normal Heart

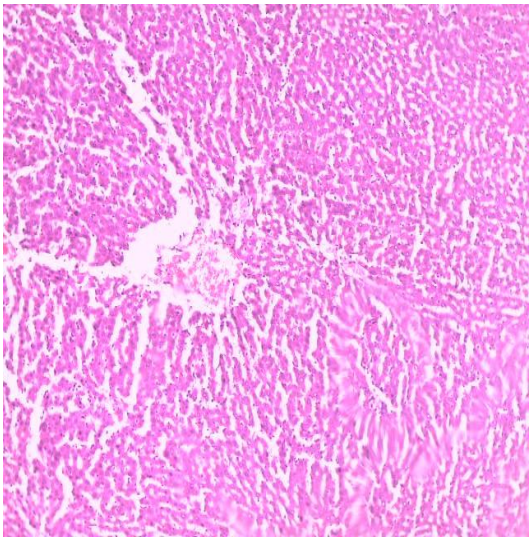


AORTA: NORMAL AORTA Liver:

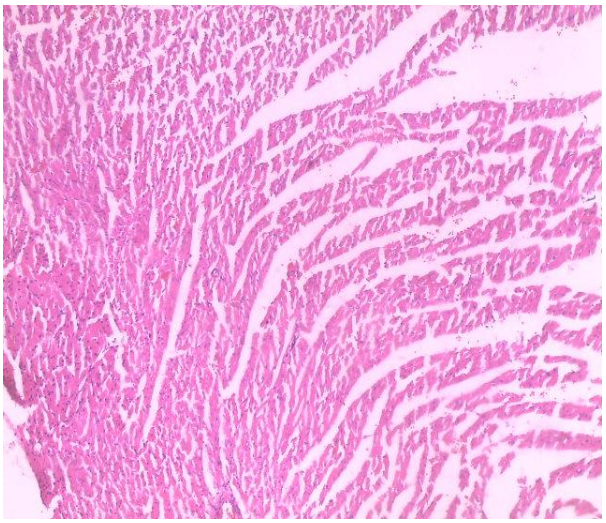


Section Of The Liver Showing Focal Area Of Chronic Inflammatory Cells Around Rge Portal Tract.

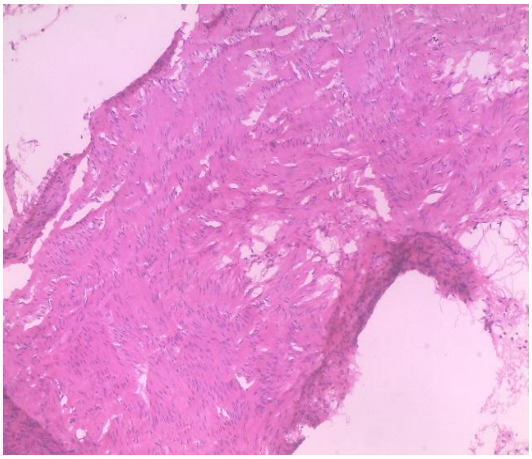
500MG/KG FEMALE



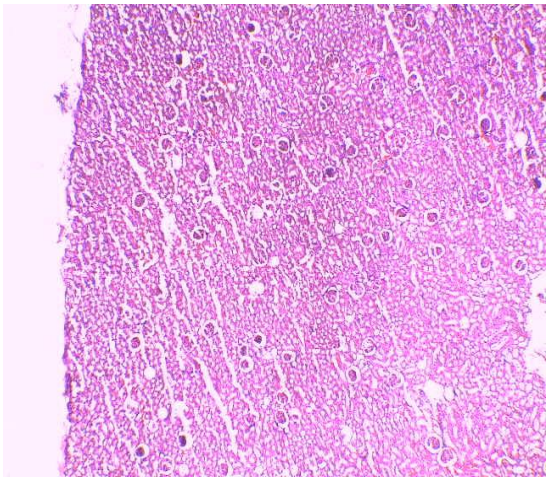
Liver: Sectiono the Liver Show Moderate Fatty Change (Steatosis)



Heart: Normal Heart

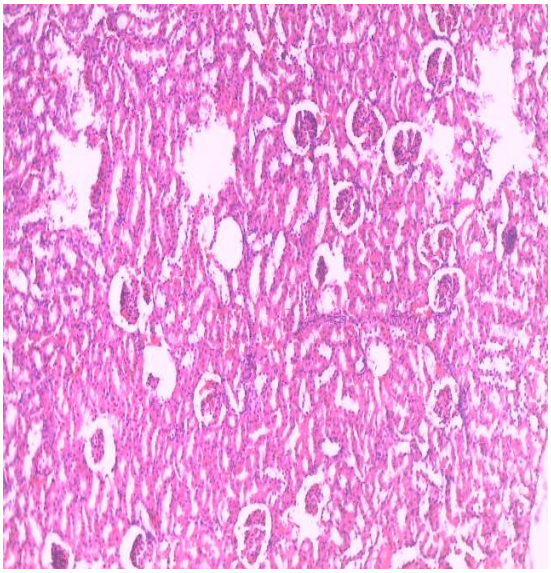


Aorta: Normal Aorta

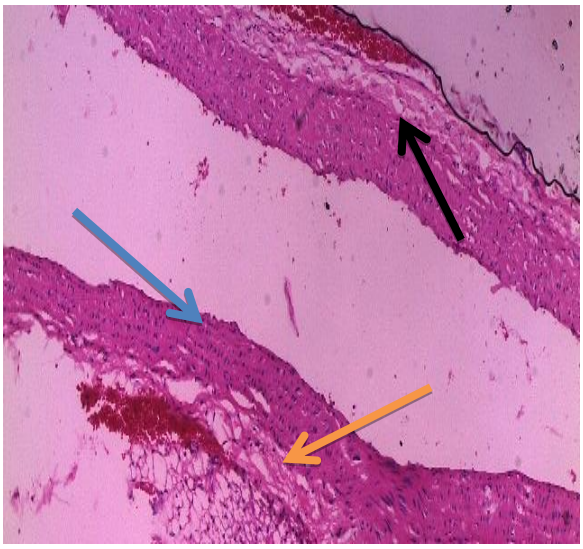


Kidney: Normal Kidney

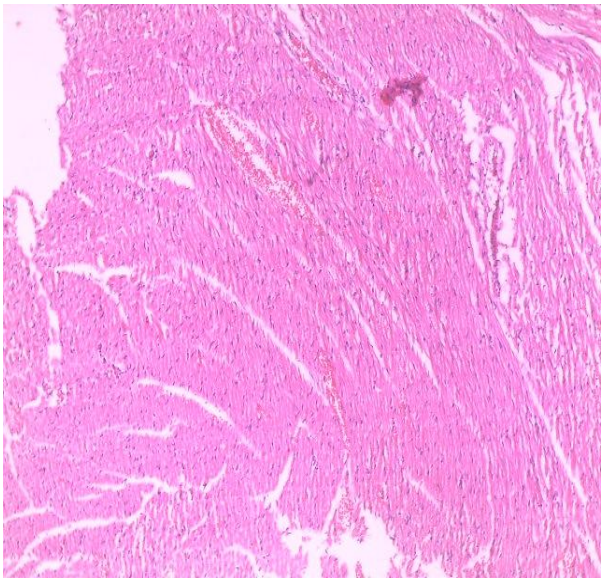
CONTROL FEMALE



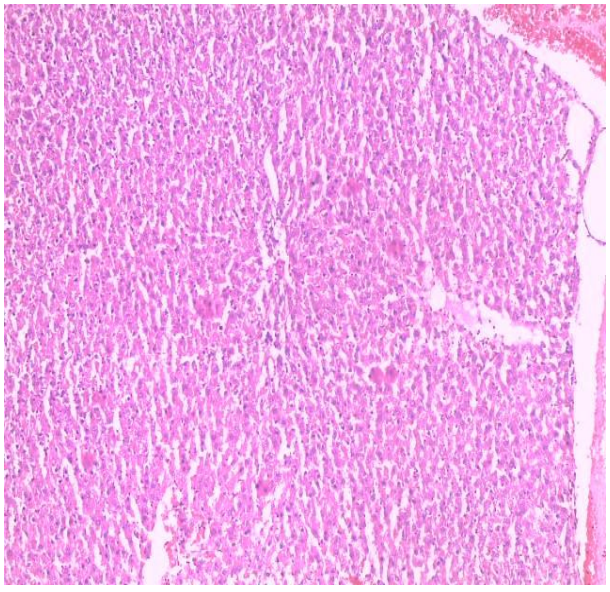
Kidney: Normal Kidney



Aorta: Normal Aortic Valve Consisting Of Endothelial Cell Layer (Blue Arrow), Interstitium(Black Arrow) and Smooth Muscle Layer(Orange Arrow)



Heart: Normal Heart



Liver: Normal Liver

CHAPTER FOUR

DISCUSSION

When screening natural products for potential pharmacological activity, it is important to first evaluate their toxicity in order to ensure the safety of the product. One way to do this is by determining the lethal dose (LD50) of the extract, isolated compounds, or formulation. This helps to provide initial information on the mode of toxic action, classifies the product for labelling purposes, and determines the appropriate dose for animal studies. If the LD50 is found to be high (e.g., 5000 mg/kg), no further acute testing will be needed. The LD50 value represents the dosage at which 50% of the mice exposed to fumarate would die. It's can be said that fumarate has a relatively low toxicity, as evidenced by the fact that even at higher doses of 1600 mg/kg and 2900 mg/kg, no mortality or toxic symptoms were observed. The LD50 value of 3,807.89 mg/kg suggests that fumarate has a relatively high safety margin in mice, meaning that mice can tolerate relatively high doses without experiencing significant toxicity.

In the male rats, the body weights were measured on the initial (0) day, 9th, 18th, and 28th days. The graph shows no significant changes in body weight across all four groups (control, fumarate at 50 mg/kg, fumarate at 150 mg/kg, and fumarate at 500 mg/kg) throughout the 28-day treatment period. This suggests that the administration of fumarate did not have a significant effect on the body weight of male rats. While In the female rats, the body weights were also measured on the initial (0) day, 9th, 18th, and 28th days. Fumarate treatment did not result in significant changes in body weight over the 28-day period.

Haematological parameters, such as red blood cell count, white blood cell count, platelet count, and haemoglobin concentration, can be used to assess the effects of drugs on blood-related

functions. The haematopoietic system is very sensitive to the presence of toxic compounds, making it an important indicator of health in both humans and animals. So, by measuring these haematological parameters, we can get an idea of how Fumarate may be affecting the body.

White blood cells (WBCs) are the body's first line of defense against infection, tissue damage, and inflammation. They circulate in the blood and patrol the body, looking for signs of trouble. Once they detect a problem, they activate and mobilize, helping to clear out the offending agents and initiate the healing process. Without WBCs, the body would be much more vulnerable to infection and other harmful conditions.The results suggest that fumarate administration led to significant changes in some white cell parameters compared to the control group. In male rats, fumarate administration resulted in a significant increase in WBC count and MON count. In female rats, fumarate administration led to a significant increase in LYM count, GRA count, and WBC count at higher doses (150 and 500 mg/kg). The percentages of LYM, MON, and GRA did not change significantly in both male and female subjects. Overall, the results suggest that fumarate administration may lead to changes in white cell parameters, which may have implications for immune function.

The drug did not significantly affect the red blood cell indices, which suggested that it did not affect the production of red blood cells, their morphology, or their ability to withstand osmotic stress. This finding is important because it indicates that the drug is unlikely to cause any adverse effects on the red blood cells. It also suggests that the drug is unlikely to cause any anemia or other blood disorders.

Serum marker enzymes are biochemical parameters associated with health indicators and have diagnostic importance in routine clinical assessment of health status. Alanine aminotransaminase

(ALT) and aspartate aminotransaminase (AST) are mainly used in the evaluation of liver damage caused by drugs or other hepatotoxins (Ramaiah, 2011)The release of ALT and AST from the liver and heart and their increased plasma concentrations are indicators of liver damage. However, ALT is more specific to the liver and is therefore a better parameter for detecting liver damage (0zer et al., 2008). It can be observed that fumarate treatment did not significantly affect albumin and total protein levels in both male and female rats, suggesting that the fumarate did not have hepatotoxic effects and may not have caused toxic effects on cardiac tissue (Crook, 2006). This protective effect is thought to be due to stabilization of the plasma membrane, which maintains the structural integrity of cells and repairs liver tissue damage (Pari and Murugan, 2004).

The near-normal levels of total cholesterol and triglycerides observed in groups treated with the substance may be attributed to the of hypolipidemic potential of Fumarate.

Fumarate treatment at different doses (50, 150, and 500 mg/kg) had varying effects on cholesterol and lipoprotein levels in rats. The results suggest that fumarate treatment may have a cholesterol-lowering effect in rats, especially in male rats. However, the effects on HDL and LDL levels may differ depending on the dose and sex of the rats.

Specifically, the groups treated with fumarate at 50 mg/kg, 150 mg/kg, and 500 mg/kg all show a significant decrease in TG levels compared to the control group. This suggests that fumarate treatment has a positive effect on reducing triglyceride levels in rats. It's important to note that the dose of fumarate appears to have an impact on its effectiveness in reducing TG levels. The group treated with the highest dose of fumarate (500 mg/kg) shows the most significant reduction in TG levels compared to the other two doses. These results suggest that fumarate

treatment, particularly at higher doses, can effectively lower triglyceride levels in rats. However, further research and investigation are needed to fully understand the mechanisms and potential benefits of fumarate treatment in reducing TG levels.

Based on the results gotten from histopathological examination, it shows that the control female and male rats showed normal characteristics in the kidney, aorta, heart, and liver.

Histopathological studies of the kidneys, aortas and heart sections of rats treated with doses of 50mg/kg, 150 mg/kg and 500mg/kg showed no significant microscopic changes when compared with the controls group (both male and female rats). In the treated rats of liver sections revealed moderate to severe fatty change (stealtosis). This suggests that there is an abnormal accumulation of fat within the liver. This suggests that Fumarate is hepatotoxic. The hepatoxicity may limit its therapeutic potential and require careful monitoring for liver damage.

CHAPTER FIVE

CONCLUSION

The findings suggest that fumarate has relatively low toxicity in mice, with a high safety margin at doses below 3,807.89 mg/kg.

In conclusion, fumarate has potential therapeutic benefits, such as cholesterol-lowering and immune modulation effects. However, careful monitoring of liver function and dosing guidelines are recommended to avoid hepatotoxicity. Further research is needed to fully understand the potential benefits and side effects of fumarate treatment and its applicability to humans.

5.1 Recommendation

Based on the findings of the study, it can be recommended that Fumarate has a relatively low toxicity and can be tolerated at high doses without significant adverse effects. However, it may lead to changes in white cell parameters and liver function parameters, particularly in female rats, and can cause fatty liver changes.

Further research is needed to understand the mechanisms of these effects and to determine the potential risks of fumarate treatment in humans. It is recommended that future studies investigate the long-term effects of fumarate treatment on both male and female subjects, and examine its potential benefits in treating specific conditions such as high cholesterol and triglyceride levels. Additionally, further investigation into the potential hepatotoxicity of fumarate is needed, and careful monitoring for liver damage should be done in any future clinical trials.

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