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Physiological and Histological Study on Liver of Male Albino Rats Treated with Retinol Drug (Retan)

A Thesis

Submitted to the Council of College of Science / University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

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صدق الله العلي العظيم

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supervisors Certification

I certify that this thesis entitled **(Physiological and Histological Study on Liver of Male Albino Rats Treated with Retinol Drug (Retan))** was carried under our supervision at the department of Biology, college of Science, University of Babylon as partial requirement for the degree of Master philosophy in Biology. Accordingly, I recommended this study for discussion.

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CERTIFICATION COMMITTEE

We, are the examining committee, certify that we have read this thesis entitled **" Physiological and Histological Study on Liver of Male Albino Rats Treated with Retinol Drug (Retan) "** and examined the student **" Zainab Mohammed Abbas "** in its content. In our opinion, it meets the standards of a thesis for the degree of Master of Science in Biology and accepted with "**Excellent**" degree.

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DEDICATION

To the most beautiful soul who left the world, the one whose name we knew better than our own, and those were the first testimonies of our victory to me

father who did not complete the journey with me.
To the one who supported me in her prayers and supplications, and stayed up at night to illuminate my path, to the source of kindness and tenderness, to the most beautiful smile in my life, to the most wonderful and strong woman in

existence, my beautiful moon, my mother.
To the dearest creature to my heart, my beloved, my soul, my grandmother.
For those who love me, for those who rejoice with me and are proud of me and
eagerly await my coming when I am gone... my brothers.
To my dear homeland, I am proud and proud ... I dedicate the fruit of my effort.

Zainab

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Zainab

Summary

The current study aime to know the effect of using different doses of Isotretnion (Iso) drug in different periods. The study was achieved by studying the effect of drug on some blood indices (WBCs, LYM%, Mid%, Gran%, RBCs, Blood hemoglobin (HGB), HCT, MCV, MCH, MCHC, ALP, AST, ALT), some biological marker of liver damage (FAP1, Kallistainen) and histological changes of liver tissue.

The current study attended for the period between November (2021) to June (2022) at the animal house that belongs to the science department, Babylon university. Forty white albino male rats were used, divided into eight groups (n = 5) treated for 30 days in the following arranges: group 1 (G1) treated with water (negative control), group 2 (G2) treated with canola oil (positive control), groupe 3 (G3), group 4 (G4), group 5 (G5) treated with 20 mg /kg for 24 hrs ,48 hrs, and 72 hrs respectively and group 6 (G6), group 7 (G7) and group 8 (G8) treated with 40 mg /kg for 24 hrs ,48 hrs and 72 hrs respectively.

The results showed that the white blood cell counts decreased significantly ($p \leq 0.05$) in G5 and G6, also the lymphocyte percentage decreased significantly ($p \leq 0.05$) in G4 and G6. Blood hemoglobin decreased significantly ($p \leq 0.05$) in G6, the Mid% showed significant increased ($p \leq 0.05$) for treated groups. Red blood cells count, Hematocrit, mean corpuscular volume, Hemoglobin, mean corpuscular hemoglobin and Mean corpuscular hemoglobin concentration. The results of liver enzyme (Alanine transaminase, an alkaline aminotransferase, Aspartate phosphatase) increased significantly($p \leq 0.05$) for both doses and for all time periods. also showed Kallikrein-binding protein reased significantly ($p \leq 0.05$). While FABPI Fatty acid binding protine index, the results showed that its level increased significantly ($p \leq 0.05$) for both doses and for all time

periods. Blood hemoglobin decreased significantly ($p \leq 0.05$) in G6, the Mid% showed significant increased ($p \leq 0.05$) for treated groups. Red blood cells count, HCT, MCV, HGB, MCH and MCHC. The results of liver enzyme (ALT, AST, ALP) increased significantly ($p \leq 0.05$) for both doses and for all time periods.kallistainen also showed decreased significantly ($p \leq 0.05$).While FABPI index ,the results showed that its level increased significantly ($p \leq 0.05$)for both doses and for all time periods.

Photomicroscopes of rat's liver treated with(20mg/kg) for 24 hrs showed variable changes during a different of the study cross section rat liver showed treated with isotretinoin group 20mg/kg for 24 hrs normal hepatocytes while some bi-nucleated cells refer to regeneration. Photomicroscopes of rat's liver treated with(20mg/kg) for 48 hrs cross-section of the liver tissue of male rats is showed as indicated by the presence of the central vein, hepatocytes, and sinusoids. Photomicroscopes of rat's liver treated with(20mg/kg) for72hrs cross-section of the Liver tissue of male rats is showed central vein congested, K-cell proliferation, cellular swelling associated with hydropic degeneration. Photomicroscopes of rat's liver treated with (40mg/kg) for 24hrs cross section rat liver showed treated with isotretinoin dilated central vein, dilated, congested sinusoids. Photomicroscopes of rat's liver treated with (40mg/kg) for 48 hrs cross section rat liver showed treated with isotretinoin central vein congested, prominent K-cell ,angiectasis, Photomicroscopes of rat's liver treated with (40mg/kg) for 72hrs cross section rat liver , showed treated with isotretinoin herniated central veins, These effects showed due to the administration of isotretinoin in different doses and durations compared to the group treated with oil show up marked fatty change and dilated sinusoids.

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List of abbreviation

Abbreviation	Description
1GF-1	Insulin growth factor-1
ALP	An alkaline phosphatase
ALT	Alanine aminotransferase
AMH	Anti-Mullerian hormone
APCs	antigen presenting cells
AST	Aspartate transaminase
ATRA	Trans-retinoic acid
AV	Acne Vulgaris
CVD	The chemical vapor deposition
ECM	Extracellular matrix
FDA	The US Food and Drug Administration
FoxO	Nuclear protein transcription factor O
FSH	The follicle stimulating hormone
GGT	Glutamyl transferase
Gran%	Granulocyt %
HCC	Hepatocellular carcinoma
HCT	Hematocrit
HDL	High-density lipoprotein
HGB	Hemoglobin
HSCs	Hepatic stellate cells
IBD	Inflammatory bowel disease
ISO	Isotretinoin
LCFA	Long-chain fatty acids

LDL	Low –density lipoprotein
L-FABP	Liver –type fatty acid –binding protein
LH	Luteinizing Hormone
LSEC	liver sinusoidal endothelial cells
LYM%	Lymphocytes
MCH	Mean corpuscle hemoglobin
MCHC	Mean corpuscle hemoglobin concentration
MCV	Mean corpuscle volume
Mid%	Mid –range absolute count percentage
NAFLD	Non-alcoholic fatty liver disease
NGAL	Neutrophil gelatinase-associated lipocalin
NKT	Natural killer T
NPCs	numerous non-parenchymal cells
PLT	Platelet count
PPARα	Peroxisome proliferator-activated receptor-α
RA	Retinoic acid
RBC	Red blood cell
WBC	White blood cells

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Isotretinoin (ISO) is the most effective treatment currently available for acne. Isotretinoin or 13-cis-retinoic acid is recommended for severe inflammatory acne of the streptococcus or lumpy types and for acne that has proven resistant to previous treatments with antibiotics or topical medications (Marie et al., 2022). Since 1933, the retinoid ISO has been routinely utilized in the treatment of severe cystic and chronic acne vulgaris. The usage of ISO is growing by the day; nevertheless, there are a number of case reports in the literature about its negative effects, and further research is needed (Doğan and Aghayarov,2022).

Iso has revolutionized the treatment of acne vulgaris, and it's becoming more well acknand knowledge as a viable therapy choice for a variety of other dermatological disorders. Forbat et al. (2018) examine the evidence supporting the use of iso for a variety of dermatological indications, including hidradenitis suppurativa, sebaceous gland pathology, rosacea, and scarring alopecia, cosmetic dermatology, and non-melanoma skin cancer prophylaxis, and consider alternative uses within dermatology practice. Isotretinoin was found to be beneficial in the research, however most of them lacked statistical power and were often confined to case studies. Isotretinoin is a well-tolerated medicine that can be used as an adjuvant treatment or as a second-line agent in resistant instances who haven't responded to first-line therapy when administered in the right cohort and with proper prior counseling about adverse effectcs.

Drug-induced liver damage is a rare but significant and difficult kind of liver disease. The rationale for this is that medication-induced liver damage can be serious, even deadly, but it can typically be reversed by stopping the offending drug. Abrupt hepatitis, chronic hepatitis, acute liver failure, biliary blockage, and fatty liver disease are all symptoms of drug-induced liver harm. Even the most committed

subspecialist in the field finds it difficult to keep track of which medications induce liver harm and what pattern is characteristic of each agent, drug-induced liver impairment is becoming a more serious problem (Hong et al ,2022).

Aspartate transaminase (AST) and Alanine aminotransferase (ALT)are two aminotransferases, Liver enzymes, such as (AST) and (ALT), are associated with not only the development and prognosis of hepatocellular carcinoma and liver metastasis but also other types of cancer. In patients infected with the hepatitis C virus, elevated ALT is considered to be an independent risk factor for hepatocellular carcinoma) Kobayashi et al.,2022). Alkaline phosphatases (ALP) are widely distributed enzymes (e.g., liver, bile ducts, intestine, bone, kidney, placenta, and leukocytes) that catalyze the release of orthophosphate from ester substrates at an alkaline pH (Moura et al.,2022).

Alkaline phosphatase belongs to a group of zinc metalloenzymes that are abundant in the microvilli of the bile canaliculus, as well as other tissues (e.g., bone, intestines, placenta), children and adolescents have raised levels of ALP throughout growth due to increased osteoblastic activity. Females' typical reference range levels likewise rise with age (Metra et al ,2022). Fatty acid-binding protein (FABP) was first discovered in 1970, FABP can bind to long-chain fatty acids and certain other lipids in various tissues including the mammalian adipose tissue, intestinal mucosa, muscle, myocardium, liver, and kidney. (Sasayama et al., 2021).

Kallistatin (kallikrein-binding protein) is a newly discovered serine protease inhibitor that is generated and expressed mostly in the liver and transported throughout the heart, kidneys, and blood vessels (Chao et al,2018). Kallistatin has been shown in several trials to be an excellent biomarker for the early identification of liver fibrosis in a variety of liver disorders (Hasan et al ,2019). Kallistatin levels were found to be considerably reduced in patients with liver fibrosis.It was

demonstrated that even a single biomarker level assessment may diagnose patients in the early stages of liver fibrosis with a sensitivity of 96.7 percent and a specificity of 50 percent (Zhelezniakova et al,2021).

1.2 Aim of the study

Iso used for the treatment of dermatologic conditions. Although it has broad side effects due to the lack of studies on the periods and doses in which the drug is administered, this experiment is designed to achieve by the studying the following objectives:

1- Some blood parameters.

2- Liver enzymes ALT, AST and ALP.

3- Some biomarkers for liver damage such as kalistainen and FABP1.

4- Histopathological study of liver.

CHAPTER TWO
LITERATURE REVIE

2. Literature Review

2.1. Liver

Liver is a complex organ composed of an organized network of heterogeneous cells essential maintaining homeostasis. The liver lobules are composed of parenchymal cells such as hepatocytes and numerous non-parenchymal cells (NPCs), including lymphocytes, antigen presenting cells (APCs), stellate cells, and liver sinusoidal endothelial cells (LSECs) (Candarlioglu et al., 2022).

The liver vasculature is an anatomically specialized structure where oxygenated arterial and nutrient-rich portal venous blood mix within a sinusoidal space that drains into a hypoxic central vein. The sinusoids form the fundamental functional unit of the liver and are lined with unique endothelial cells known as LSECs. Although their physiology is essential in key aspects of liver biology, the identification of LSECs is not straightforward, it requires tracking multiple cellular and phenotypic markers despite no standardized agreement within the field (Suckow et al., 2019).

El–Razik et al. (2022) exogenous and intrinsic substances, foreign particles, and hormones are altered by the liver to make them less toxic or physiologically active, Haptic stellate cells (HSCs) are another important type of cell in the liver. They are mostly found in the Dissé vacuole between sinusoidal endothelial cells and hepatic epithelial cells, and they contribute about 5% to 8% of the liver's volume. HSCs are quiescent, painless cells that retain multiple lipid droplets of vitamin A, and constitute the body's largest source. This vitamin under homeostatic conditions (Tsuchida and Friedman,2017).

The function and role of quiescent hepatic stellate cells is unclear. Recent evidence suggests a role as a liver-resident antigen-presenting cell, presenting lipid

antigens to and stimulating proliferation ofNatural killer T (NKT) cells (Winau et al.,2007). When the liver is damaged, stellate cells can change into an activated state. The activated stellate cell is characterized by proliferation, contractility, and chemotaxis. This state of the stellate cell is the main source of extracellular matrix production in liver injury, this attribute makes it a key factor in the pathophysiology of the liver (Fasbender et al., 2016).

Kupffer cells are resident liver macrophages and play a critical role in maintaining liver functions. Under physiological conditions, they are the first innate immune cells and protect the liver from bacterial infections (Albuquerque and Sahingur,2022). They are responsible for the elimination of particles, immunological complexes, dormant red blood cells, and endotoxins from tissues, as well as the existence of compensatory hepatocyte hyperplasia, which can lead to end-stage liver disorders such as cirrhosis and hepatocellular cancer (Forner et al.,2018). As a result, the liver serves as an essential immunological location for cytokine signaling and acute-phase protein synthesis, aiding in the preservation of the balance between immune surveillance against infections and tolerance to harmless antigens and commensal microorganisms make up the majority of the ECM in the liver in its natural condition (Kubes and Jenne, 2018).

Over 500 different tasks, including immunological, metabolic, and synthetic, are carried out by collagen, glycoproteins, proteoglycans, and hyaluronic acid. It is composed of repeating functional tissue units and has a hierarchical structure. It controls the bulk of chemical levels in the blood leaving the stomach and intestines, exposing them to a variety of nutrients, pathogens, endotoxins, and microbial components that enter the portal vein from the digestive system (Williams et al.,2017).

The liver performs many tasks, including the removal of foreign and endogenous toxins and the production of essential proteins such as blood clotting factors and albumin. Some of the vital functions of the liver include blood purification, the formation of essential blood clotting like heparin and other proteins, prescription drugs and nutrients, and the preparation of waste products, it is also essential for the metabolism of proteins, lipids, and carbohydrates, for the delivery of vitamins, for the conjugation and excretion of bilirubin in the bile, for the activation of glycogen, and triglyceride and mineral reserves, and for the synthesis of bile salts (Jenkins et al., 2010; Huang et al., 2014; Suckow et al, 2019).

It is also essential for the metabolism of proteins, lipids, and carbohydrates, for the delivery of vitamins, for the conjugation and excretion of bilirubin in the bile, for the activation of glycogen, and triglyceride and mineral reserves, and for the synthesis of bile salts (Jenkins et al, 2010; Huang et al., 2014; Suckow et al, 2019).The liver is involved in the metabolism and removal of toxicants from the body, and its histologic and biochemical characteristics are used to determine chemical toxicity (Rahman et al,2022).

2.2. Retinol (Isotretinoin)

Isotretinoin with a molecular weight are 300.44 and the molecular formula (2 C20-H28-O2)] is a retinoid and a synthetic vitamin A (retinol), a lipophilic vitamin, is essential for brain development and function throughout life. It works by binding to nuclear receptors and modulating gene transcription through its active metabolite, retinoic acid (Marie et al., 2022).

The Food and Drug Administration (FDA) was first approved oral Iso for the treatment of severe acne in 1982 after it was developed in 1955. Even now, Iso is a very effective treatment for moderate, severe, and recurrent acne, continually

improving the condition of the skin after treatment. The only acne treatment that addresses all of the key etiological factors is Iso, but its use is restricted by a number of side effects (Abaunza,2022). One of the most alarming issues is the possibility of teratogenic effects. According to research, long-term highdose use of retinoid-containing medicines can cause liver dysfunction without causing liver damage. Hepatic impairment can occur in as many as 15% of isotretinoin users (Kaźmierska et al.,2022).

Finding beauty has become an obsession for many individuals. Everyone, especially teenagers and young people, seeks beauty; it has no relation to age, gender, color, physical form, or size. Isotretinoin, a vitamin A derivative, is one of the ingredients in most cosmetic, hair, and skincare products. Severe inflammatory acne, especially nodulocystic acne, and acne that has resisted prior antibiotic or topical therapy are recommended to be treated with isotretinoin. It controls keratinization in the sebaceous glands by interacting with specific retinoid receptors and changing the transcription of certain genes (Vieira et al, 2012).

It reduces keratinization of hair follicles, inflammatory cytokine activity, and sebaceous gland enlargementIso also reduces the quantity of Propionibacterium acnes bacteria (Okan Kizilyel et al,2014). This particular vitamin A derivative may alter the liver by raising the blood levels of a liver enzyme and altering the levels of certain lipids such as triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol (Kaymak and Ilter,2006).

2.2.1. Description

Merritt et al (2009) disclosed that a retinoid comes in (10-mg, 20-mg, and 40-mg) soft gelatin capsules for oral use in Accutane, as well as parabens (methyl and propyl), hydrogenated soybean oil flakes, and the color systems stated below; Red

iron oxide in amounts of 10 mg. 20 millimeters FD and C Yellow No. 6, FD and C Yellow No. 10, and FD and C Yellow No. 6 Titanium dioxide, FD and C Red No. 3, and FD and C Blue No. 1; 40 mg A mineral that occurs naturally is titanium dioxide.

2.2.2. Side effects

Unlike other prescription drugs for severe acne, iso is the most costeffective. However, it has been linked to many significant and disfiguring side consequences that patients need to be aware of (Jarab et al.,2022). Of those between the ages of 12 and 25 who have acne vulgaris (AV), over 85% do so, and also affects younger children) Lynn et al., 2017). It is approved asthe tried-and-true first-line therapy for moderate to severe acne. Responding to previous treatments, however, it has a variety of adverse effects that vary from minor to life-threatening (Brzezinski et al,2017).

Up to 35 percent of newborns exposed to the drug in utero are expected to have congenital malformations, while 30-60 percent of infants exposed to Iso before birth have neuropsychiatric defects (Choi et al., 2013). Conversely, most patients are unaware of there are just warnings in the product brochure regarding the possible side effects of Iso and how to manage them. There are no further risk mitigation techniques. More than half of patients in a recent Saudi study did not understand hyperlipidemia as a negative. Isotretinoin impact Furthermore, more than a third of them were ignorant of the fact that isocan elevate liver enzyme levels, and in the second study, more than half of the patients were ignorant of the fact that it is not advisable for them to give blood while taking it. They were not aware that iso should be discontinued at least 1 month before conception (Iman et al., 2021).

According to new research, patients' understanding of the need for contraceptive use among,only a small number of women who are of reproductive age who take iso use contraception as directed, which is insufficient.The lack of iso therapy's expected side effects highlights the need to increase patient understanding of the drug and its proper administration. This study, which assesses patients' understanding of Iso usage recommendations and associated adverse effects, is the first of its type in Jordan, by increasing patient awareness and information regarding the use of Iso, the study's findings are expected to help better understand the local situation and develop interventions aimed at improving patient outcomes (Jarab et al, 2022).

2.2.3. Isotretinoin's mode of action

Due to its potent abilities to suppress the release of sebum, which is predominantly produced by the death of fat cells, isois often regarded as the most effective treatment for severe kinds of acne vulgaris. Young-onset neuroblastoma, myeloid leukemia, and basal cell carcinoma can all be treated with iso thanks to its apoptotic impact. Primary human keratinocytes, B16F-10 melanoma cells, adult T-cell leukemia cells, and Dalton lymphoma ascites cells all experience apoptosis as a result of exposure (Melnik, 2017). The mechanism of action of isotretinoin has been the subject of numerous studies, some of which have been successful in illuminating these pathophysiological pathways. Nelson et al. (2006) discovered that the proteins linked to tumor necrosis factor-associated apoptosis (TRAIL) and neutrophil gelatinase associated lipocalin (NGAL) contribute to iso ability to produce apoptosis and cell cycle arrest in human SEB-1 fat cells.

In 2016, Kelhälä et al. discovered that TRAIL and NGALare more expressed in the skin of acne patients undergoing iso medication. TRAIL has been demonstrated to cause apoptosis in a number of cancer cell lines, although research has shown despite being present in many human organs, it is largely non-toxic to normal cells.

Nuclear protein transcription factor O (FoxO), particularly FoxO3a, is crucial for increasing TRAIL expression in healthy individuals (Zhang et al., 2011). Growth factor signaling controls FoxO activity in acne vulgaris. This indicates how FoxO phosphorylation followed by nuclear FoxO suppression via Cytoplasm sequestration via 14-3-3 binding can occur when insulin growth factor-1 (IGF-1) and insulin, which are high during puberty or in hyperglycemic patients who consume a lot of dairy products (Mordoret al., 2002).

Isotretinoin is completely converted to trans-retinoic acid in fat cells (ATRA) (Wang et al.,2014). Studies show that iso-treated acne patients had higher levels of TRAIL in their sebaceous glands, suggesting that this medication may increase FoxO/TRAIL signaling in these glands, resulting in the death of sebaceous cells. The most significant anti-acne effect of systemic isotretinoin therapy in acne patients is lipid-lowering as a result of this mechanism (Melnik,2017).

Another FoxO-dependent pro-apoptotic protein that is enhanced by isotretinoin treatment and increases in human fat cells following isotretinoin treatment is IGF-binding protein-3 (IGFBP3). RXR and RAR (retinoid X receptor and retinoic acid receptor, respectively) are nuclear transcription factors that interact with IGFBP3 (Sakoe et al, 2010). As a result, the contact between RXR and IGFBP3 participates in RXR's transcriptional activity, modulating IGFBP3's effects on cellular death (Schedlich et al,2007). Draghici et al, (2021) RAR activation induced by FoxO-dependent nuclear IGFBP3 upregulation may lead to apoptotic NGAL overexpression. All of these data support the idea that iso has a role in promoting the creation of proteins that promote apoptosis TRAIL- Fox O- IGFBP3 and NGAL.

2.2.4 Effect of Isotretinoin on The Liver Enzyme

The effects of Iso on liver enzymes and lipids have been studied in the literature, and it has been suggested that oral Iso may lead to changes in the liver. AST and ALT aminotransferases, TGs, HDL, and to varying degrees LDL (Vieira et al.,2012). The texture of the liver tissue is affected by Iso Infiltration of inflammatory cells surrounding the central vein was seen, leading to the conclusion that long-term exposure to iso causes liver injury, changes in liver tissue, and affects levels of functional liver enzymes, especially at high doses disparities in life (Tawfiq et al., 2020).

These changes in lipid profile also proved temporary, returning to baseline 2 months after treatment ended. found elevated AST, ALT, and TG levels in 130 individuals treated with iso in another investigation. HDL levels at the beginning of treatment or during treatment with iso according to various researches, while patients are being treated with iso, regular laboratory tests are required due to significant changes in liver transaminases in the blood. and lipid levels, while other research suggests that the results are minor, and no lab tests are required. wanted. According to numerous researches, in the using treatment of patients with iso, regular laboratory testing is required due to significant changes in serum liver transaminase levels and lipid levels (Vieira et al, 2012).

2.3. Biomarker Damage

2.3.1. Fatty Acid-Binding Protein (FABP-1)

The human gene FABP1 produces the protein (Fatty acid-binding protein) Further names for it include liver-type fatty acid-binding protein (LFABP). All three types of cannabinoids—endocannabinoids, phytocannabinoids, and longchain fatty acids—are bound, transported, and processed by FABP1, which is largely expressed

in the liver (Schroeder et al, 2016). L-FABP, commonly known as FABP1, is a fatty acid-binding protein that also faintly exists in the kidneys and small intestine. It has liver-like properties. FABP has been linked to tissue damage in the past, including myocardial infarction and damage to other organs such as the liver, kidneys, intestines, and lungs (Kabekkodu et al., 2016).

Hepatocytes and proximal tubular cells of the kidney contain the 14 kDa protein FABP1 in their cytoplasm. FABP1 participates in fatty acid metabolism and aids in transportation Fatty acids, and their Acyl-CoA derivatives can be utilized and stored, and may help reduce lipotoxicity thanks to TGs' ability to oxidize, fuse and bind fatty acids without cytotoxicity (Wang et al., 2015). According to some research, serum FABP1 may be a potential prognostic marker for identifying liver damage in chronic hepatitis C and Non-alcoholic fatty liver disease (NAFLD), additionally, FABP1 was highlighted by Petrescu et al. in (2016). The context of fibrateinduced stimulation of the liver PPAR oxidative gene- LCFA, particularly in the presence of high glucose levels (Akbal et al, 2016).

2.3.2. Kallikrein-Binding Protein (Kallistatin)

Kallistatin (kallikrein-binding protein) is a newly discovered serine protease inhibitor that is produced and expressed mostly in the liver and transported to the heart, kidneys, and blood vessels, and this protein has anti-inflammatory, antioxidantand anti-angiogenic properties. Tumor characteristics it has been shown to be an effective biomarker for the early identification of cirrhosis in a variety of liver diseases in a number of investigations (Zhelezniakov et al., 2021).

Kallistainen concentrations have been found to change in chronic liver disorders, which may be related to decreased hepatic protein secretion activity, according to several investigations (Nallalagangula et al., 2017). Rajab et al. (2017)

Kallaistatin levels are found to be significantly reduced in patients with cirrhosis of the liver. showed that even a single assessment of the level of vital signs may diagnose patients in the early stages of cirrhosis with a sensitivity of 96.7 percent and specificity of 50 percent.

In patients with alcoholic cirrhosis, serum kallistainen levels decrease with the progression of liver parenchymal damage, and higher activity was observed in patients compared to cases of the compensated disease, where levels differed between patients without cirrhosis and patients with simple cirrhosis depending on the status of cirrhosis, the results showed a significant decrease in kallistainen levels. This explains the action of kalistainen against fibrosis. In nonalcoholic and hypertensive patients with nonalcoholic fatty liver, we detected a significant decrease in levels as the liver parenchyma changed (Chao et al., 2018).

It might be proof of kallistatin's ability to protect the liver parenchyma from pathological alterations. Kallistatin has been shown to play a preventive function in the development of obesity in people with NAFLD. Significant variations in kallistatin levels with weight gain were seen in NAFLD patients in our study. This supports the protein's role in preventing metabolic adipose tissue alterations in NAFLD patients. Another crucial point to consider is the involvement of kallistatin in CVD. Hypertension is linked to a reduction in the activity of the kallikrein-kinin system in animal hypertension models, studies suggest that endogenous kallistatin protects against vascular oxidative stress, inflammation, and fibrosis (Zhelezniakovaet al.,2021).

In individuals with liver cirrhosis, the amount of serum kallistatinis considerably lower. The degree of liver cirrhosis and disruption of normal liver function appeared to be linked to the amount of this decline. This research backs up findings, which said that Kallistatin might be used as a novel biomarker for diagnosing and assessing

the severity of human liver cirrhosis (Cheng et al., 2015). A novel therapeutic approach for the management and treatment of cirrhotic liver diseases may also be made available by kallistatin (El Dahshan et al,2019).

It is primarily generated and released in the liver and provides defense for cells and organs against oxidative damage, fibrosis, and inflammation. Its relationship to specific liver disease is uncertain, though. The severity of cirrhosis seems to be indicated by a decline in serum kallistatin levels, with the lowest values corresponding to more severe cirrhosis.This specific tissue's kallikrein exhibits anti-angiogenic, anti-inflammatory, anti-tumor, and anti-oxidant properties. Blood, atheroma, the eyes, kidneys, liver, heart, arteries, and veins are just a few places where it has been found (Wang et al.,2005).

We hypothesized that serum kallistatin levels would be a possible diagnostic for liver cirrhosis because multiple studies have demonstrated that the liver is the principal source of kallistatin synthesis and secretion. In this investigation, we looked at the correlation between blood kallistatin and cirrhosis and HCC clinical symptoms. Recent research has shown that organ damage brought on by high blood pressure is enhanced by oxidative stress, inflammation, and immunology (Li et al, 2019).

2.4. liver function

2.4.1. Aspartate aminotransferase (AST)

Aspartate aminotransferase, often known as AST, is an enzyme that is mostly present in the liver and muscles. The AST blood test analyzes the level of AST in the blood and detects its release into the bloodstream in cases of liver injury. Additionally, the test may be used to monitor or assist in diagnosing liver issues (Hinkle et al., 2014).

An increasing amount of evidence shows that measuring AST analogs in human blood can help determine the extent of damage to some of these organs. The test is used to measure liver necrosis and determine the prognosis of liver disease. It may also help identify those who currently have alcoholic liver disease. Measurement of AST enzymes in individuals with acute myocardial infarction gives diagnostic information that differs from that acquired by measuring total creatine kinase and lactate enzymes and their dehydrogenase (Nakajima et al., 2022).

2.4.2. Alanine transaminase (ALT)

The enzyme is known as ALT, Perhaps the liver is the main location of alanine transaminase. As a result of liver cell destruction, ALT is released into the blood. The ALT test determines how much ALT is present in the blood. Even before symptoms of liver illness like jaundice, a condition that causes the skin and eyes to turn yellow, high levels of ALT in the blood might point to a problem with the liver. The blood test for ALT may help with the early diagnosis of liver disease. A liver function test frequently includes a blood ALT test (University of Rochester Medical Center, 2017).

ALT activity is largely located in the liver, although it can also be present in muscle tissue, heart, kidney, brain, and adipose tissue, at a much lower level Wroblewski and Cabaud reported a colorimetric ALT test based on glutamate measurement using paper chromatography in 1957. Subsequent experiments linked the conversion step to a second event involving the conversion of lactate dehydrogenase from pyruvate to whole blood lactate, ALT activity remaining constant for 24 hrs. before rapidly decreasing. Serum ALT activity is stable for three days at ambient temperature and three weeks in the refrigerator but drops significantly after repeated freezing and thawing (Clark et al.,2003).

It is also strongly advised not to determine ALT activity in frozen samples, as this may lead to falsely low ALT activity. Differences of 10-30% were observed from day to day (Schindhelm et al., 2006).

2.4.3. Alkaline Phosphatase (ALP)

ALP is largely concentrated in the liver, bones, kidneys, and gastrointestinal tract while being present in every cell of the body. A damaged liver may allow ALP to leak into the bloodstream. ALP levels that are higher than normal may indicate liver or bone issues (Boydet al.,2022).

The presence of a bone condition or liver injury may be indicated by high levels of ALP caused by liver disease is distinct from ALP caused by bone problems. Hypophosphatemia is a rare hereditary illness that affects the bones and teeth, and low levels could signify this. Low levels may also result from inadequate diet or zinc intake (Josse et al., 2017).

CHAPTER THREE

MATERIALS

and

METHODS

3. Materials and Methods

3.1: Materials

3.1.1 Laboratory Animals

This study is conducted in the animal house in the college of Science / University of Babylon for the period from 22/11/2021 to 22/ 6 /2022. The study included 40 white male albino rats aged 2-3 months, weighing between (100 to 150 g) and at a temperature of (25±3C) and 12 hrs (light-dark cycle) and then divided into eight groups, each group containing five rats. The animals are left before treatment for two weeks to acclimatize (Council,2011).

3.1.2 Animal Groups

The animals used in the study were divided into eight groups as follows (Fig 3-1):

Group 1 (G1): Five rats were given 0.5 ml of oil every day for 30 days (positive control).

Group 2 (G2): Five rats were given water every day for 30 days (negative control).

Group 3 (G3): Five rats were given 20 mg/ kg orally of the drug dissolved in 1 ml of oil given by oral gavage every 24 hours for 30 days.

Group 4 (G4): Five rats were given 40 mg/ kg of the drug dissolved in 1ml of oil given by oral gavage every 24 hours for 30 days.

Group 5 (G5): Five rats were given 20 mg/ kg of the drug dissolved in 1ml of oil given by oral gavage every 48 hours for 30 days.

Group 6 (G6): Five rats were given 40 mg/ kg of the drug dissolved in 1ml of oil given by oral gavage every 48 hours for 30 days.

Group 7 (G7): Five rats were given 20 mg/ kg of the drug dissolved in 1 ml of oil given by oral gavage every 72 hours for 30 days.

Group 8 (G8): Five rats were given 40 mg/ kg of the drug dissolved in 1ml of oil given by oral gavage every 72 hours for 30 days.

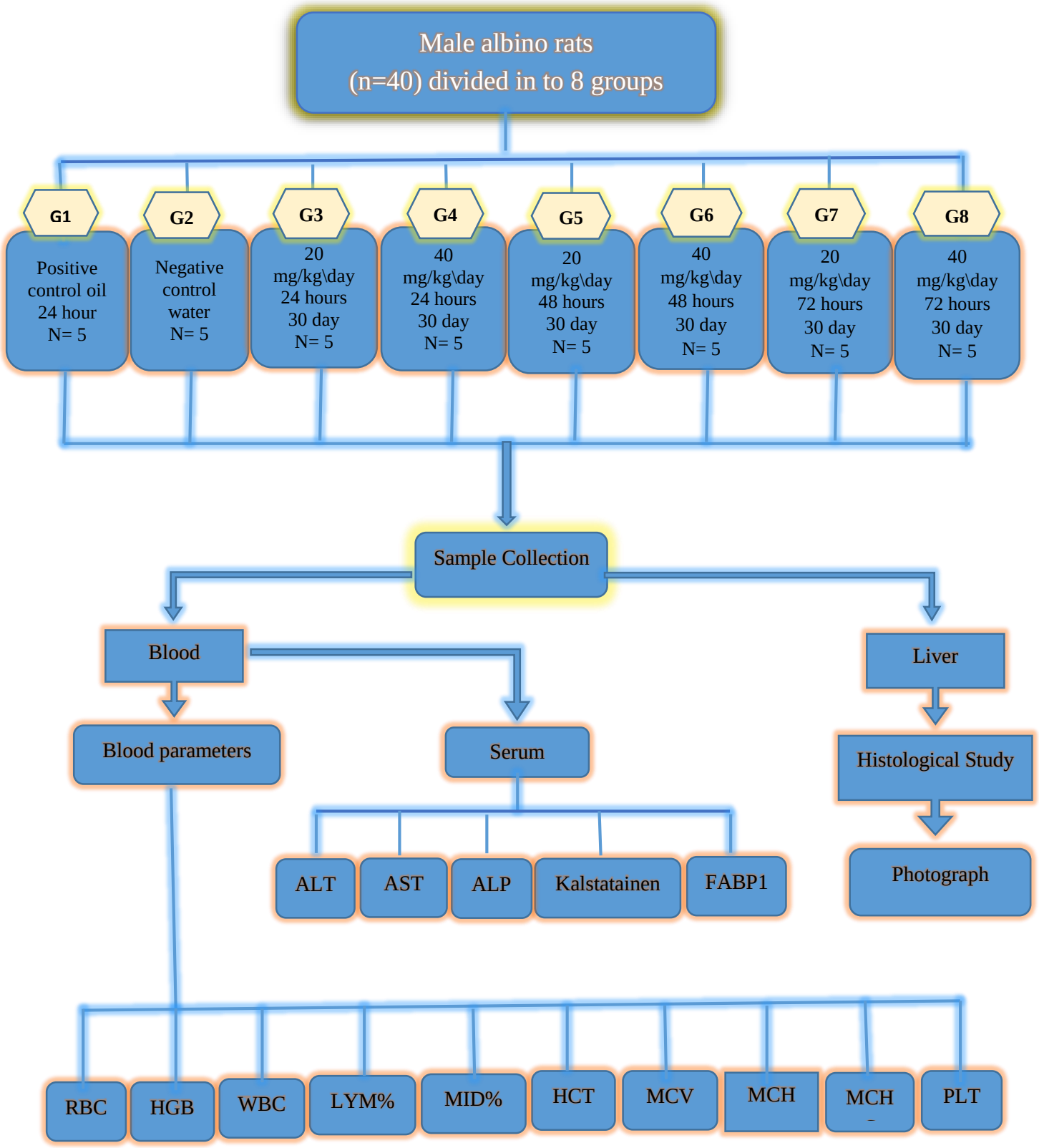


Figure (3.1): Experimental Design of the Study

3.1.3 Sample and Tissues Samples

Through the course of the study, the animals are anesthetized by chloroform and sacrificed and about 5 ml of the blood was collected directly by heart puncture with sterile syringes, then about 2 ml of blood were put in EDTA tubes for hematological parameters analysis, and 3 ml were put in gel tubes for 30 minutes at room temperature to allowing clotting, the clotted blood is centrifuged at 3000 rpm for 10 minutes and serum is collected for the estimating the liver function and Biomarker damage (ALT , AST, ALP, kallistatin,and FABP1), also,specimens of the liver is obtained for histological study.

3.1.4 Equipment and Apparatus (Appendix 1)

The instruments and their suppliers used in the current study are listed in the table (3.1).

Table (3.1): Instrumentsandtheir suppliers.

3.1.5 Chemicals, Kits and Manufactures Used in The Study (Appendix 2)

The chemicals, biologicals, and their manufacturers used in this study are listed in Table (3.2).

Table (3.2): Chemicals, biologicals, and their manufacturers used in this study.

3.2 Methods

3.2.1 Preparation of solutions

3.2.1.1 Isotretinoin Injection Dose

The drug was brought in the form of pills with a concentration of 20 mg/kg and 40 mg/kgwhich was supplied by (ERIS pharma) company,India and then the pills were dissolved in canola oil, according to the method.used in this study was

dissolved in 1 ml of canola oil. According to the method and then take the required concentration. Iso dose used in the current study was (0.6 ml) for rat weight (100-150) grams (Sanchez-Criado et al., 1999).

3.2. 2 Hematological analyses (Appendix 3)

A complete blood cell count (CBC) was assessed via the Mythic 18 VET was a fully automated hematology bench-top analyzer using impedance technology (Wassmuth et al.,2011).

3.2. 3 Liver Enzyme Function (ALT, AST and ALP) (Appendix 4)

3.2.4 Evaluation of some biomarkers of liver damage by ELISA Assay

3.2.4.1 Rat liver type fatty acid-binding protein ELISA kit Assay (Appendix 5)

3.2.4.2Rat Kallistatin ELISA Kit (Appendix 6)

3.2.5 Histological Processing and Staining (Appendix 7)

According to Kumar (2013), the normal histological treatment of the liver is prepared in order to examine any histological changes that might be present between the experimental groups and the control group.

3.2.6 Staining Procedure with Haematol xylin-Eosin (Appendix 8)

3.2.7 Microscopic Examination and photomicrography

To learn about the histological changes on the researched slides, an Olympus light microscope was used, along with a digital camera to take pictures.

3.2.8 Statistical analysis

Data was analyzed using SPSS (version 23, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard Error), and differences were compared by One-way ANOVA at $p \leq 0.05$ using least significant difference (SE) and also using

Duncan's test. The relationship between studied parameters was determined by Pearson's correlation coefficient (r).

CHAPTER FOUR

RESULTS

4. The result

4.1 Effect of Isotretinoin of Drug Does On Blood Parameters (Appendix 9)

4.2 Effect of Isotretinoin of Duration on Blood Parameters:

4.3 The effect of the duration of treatment with Isotretinoin

4.3.1 Effect of duration of isotretinoin therapy on blood parameters

The results showed when comparing the treated animals with each other depending on the duration of treatment. According to Figure 4- 1(A, B) a significant decrease ($P \leq 0.05$) in the total number of white blood cells when the rats treated with a dose of 20 mg for all periods, while the treatment with a dose of 40 mg/kg showed no significant ($P > 0.05$) in the animal treatment at period 48 hrs, compared to 72 and 24 hrs.

While the results of (4-2 (A, B show in a significant increase ($\leq P$ 0.05) in Lymphocyte when treated with a dose of 20 mg for the time period 72 hrs compared to the time periods 24 and 48 hrs, while the treatment with a dose of 40 mg showed a significant decrease ($P \leq 0.05$) in the time period 48hrs comparison 72 and 24 hrs.

While Figure (4-3)A, B showed a significant decrease ($P \leq 0.05$) of Mid% when treated with dose of 20 mg/kg and for the time period 72 hrs compared to 24 and 48 hrs, while the results of treatment with 40 mg/kg showed a significant increase ($P \leq 0.05$) for the time period 48 compared to 72 and 24 hrs Figures (4-4)A, B showed a significant decrease($P \leq 0.05$) in Granulocytes when treated with20 mg/kg in the time period, 72 compared to the time periods 24 and 48 hrs While at the dose of 40 mg /kg , it decreased ($P \leq 0.05$) significantly at the time of 24 hrs, and also increased ($P \leq 0.05$) significantly at the time period of 48 hrs, while the time period of 72 hours was not significantly ($P \geq 0.05$) affected.

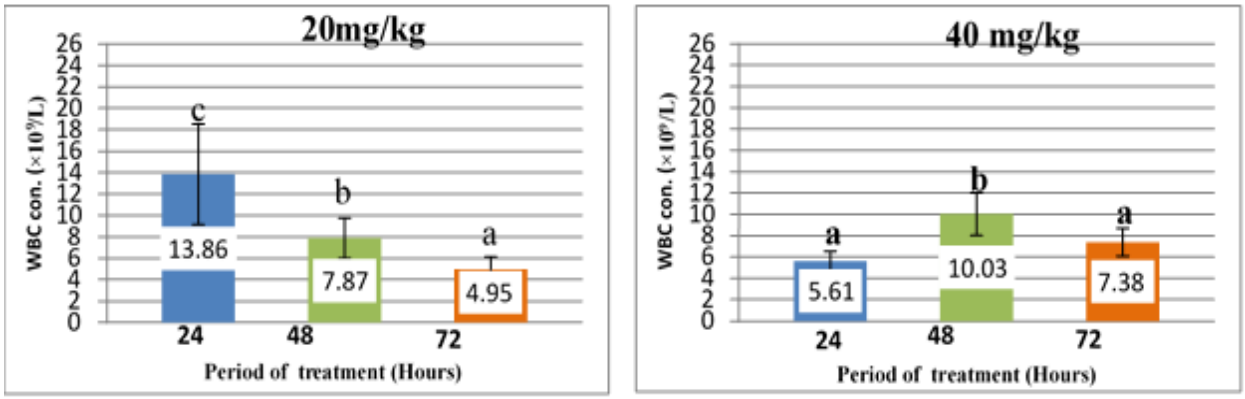


Figure 4-1(A, B): WBCs level in male rats treated with Isotretinoin (20 and 40) mg/kg in different period for 30 days, n=5.A(20),B(40) , Different letters mean there are moral differences Similar letters mean there are no moral differences.

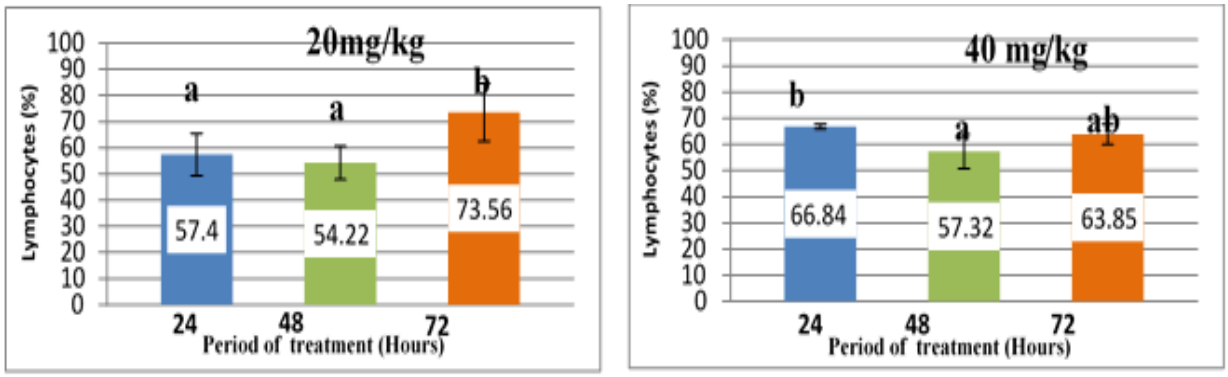


Figure 4-2(A, B): Lymphocyte in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5.A (20mg/kg) B (40mg/kg) , Different letters indicate a significant difference Similar letters mean there are no significant differences, LSD =0.05.

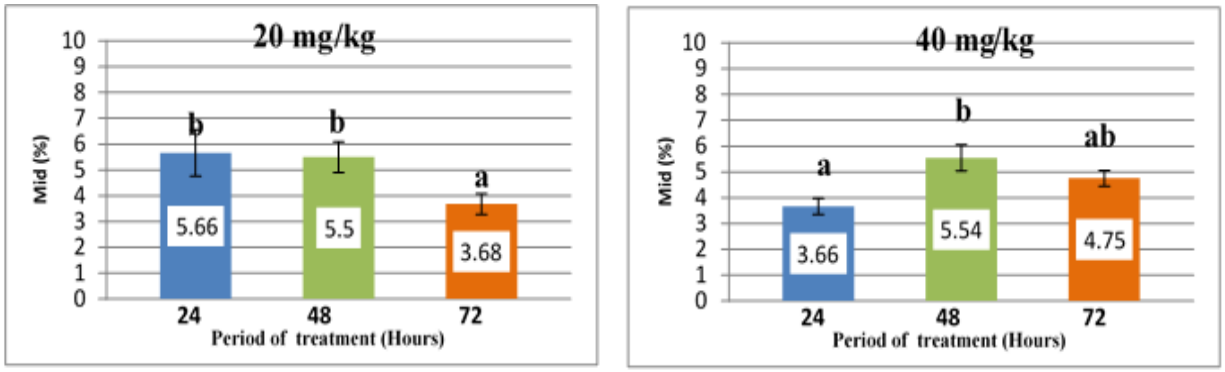


Figure 4- 3 (A, B): Mid in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5.A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD= 0.05

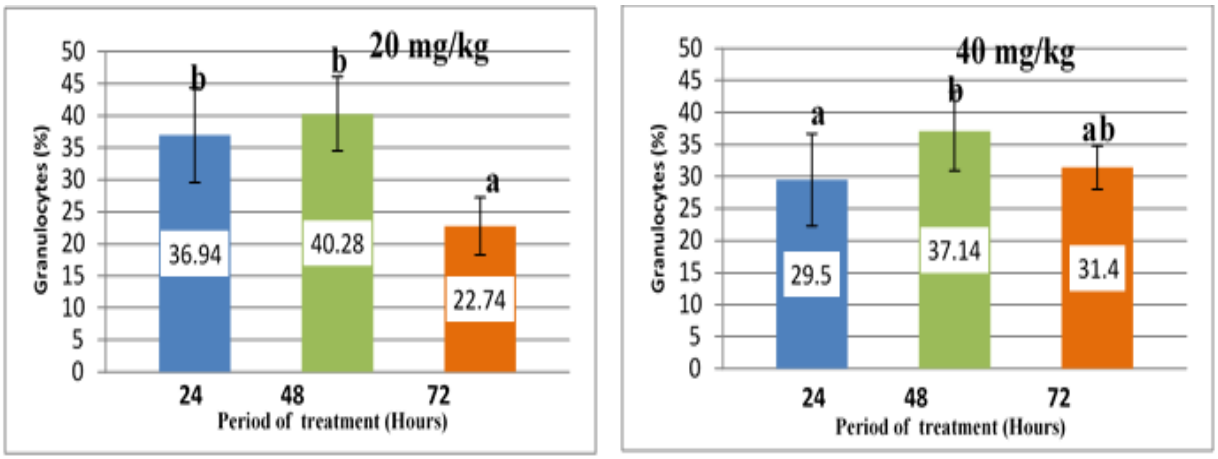


Figure 4- 4 (A, B) : Graulocytes in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days,n=5 A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

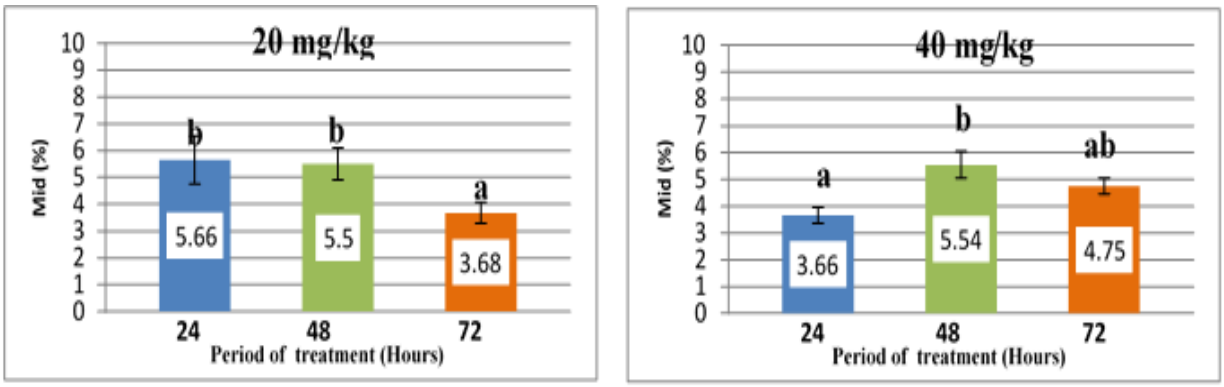


Figure 4- 3 (A, B): Mid level in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5.A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.

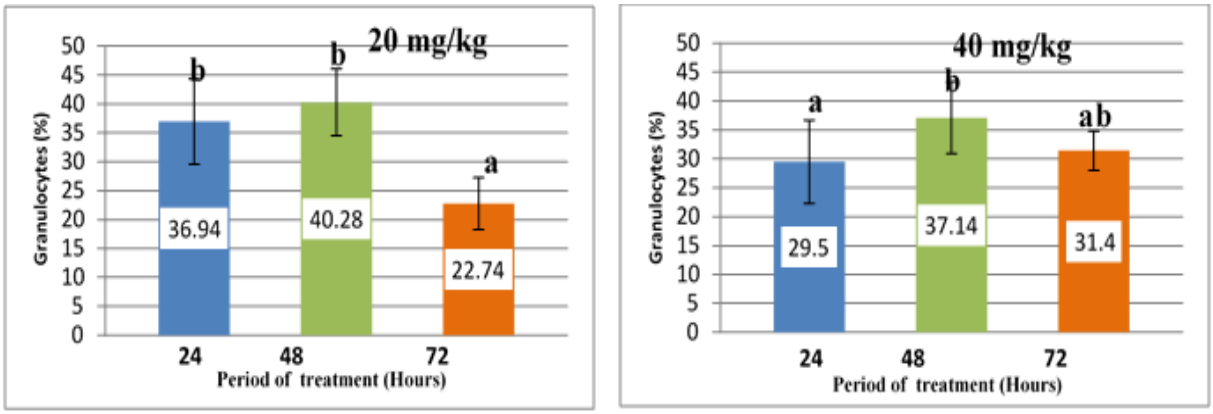
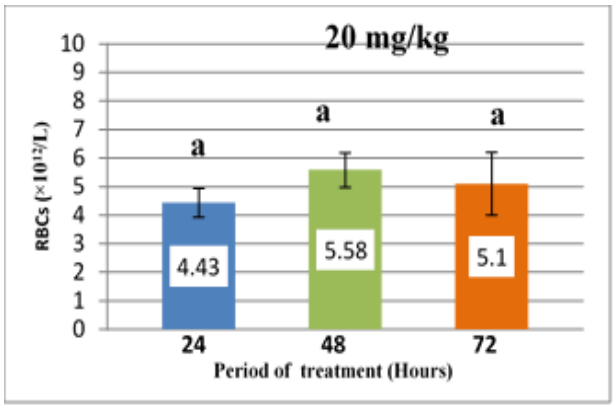


Figure 4- 4 (A, B) : Graulocytes in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days,n=5 A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

Figures (4-5A, B) showed RBCs that there were no significant (P>0.05) differences for both doses and time of treatment. While figures 4-6 (A, B) showed HGB a significant increase ($P \leq 0.05$) at the dose of 20 mg for a period of 72 hours, while it decreased significantly ($P \leq 0.05$) at a dose of 40 mg/kg for a period of 24 hrs figures 4 -7 (A, B) also showed for the blood HCT, there were no significant

(p>0.05) differences when treated with 20 mg /kg, but it showed a significant decrease (P<0.05) when treated with 40 mg/kg for 24 hrs compared to the 48 and 72 hrs.



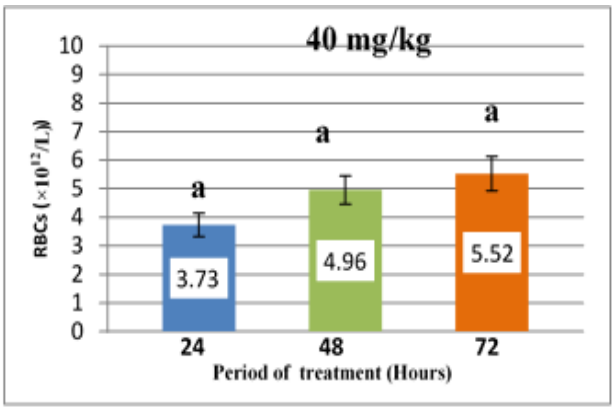


Figure 4-5(A, B): RBCs level in male rats treated with Isotretinoin (20 and 40) mg/kg in different periods for 30 days, n=5 A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

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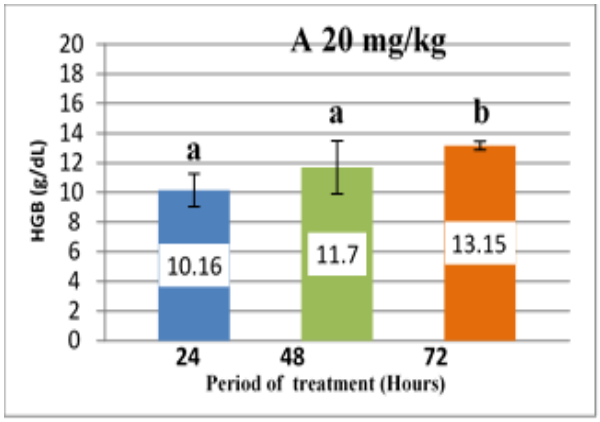


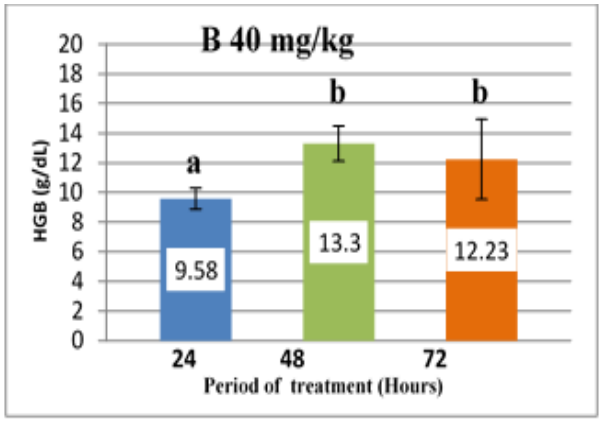
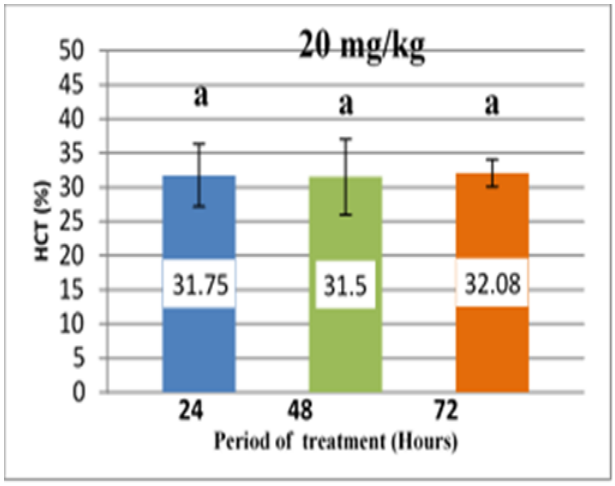
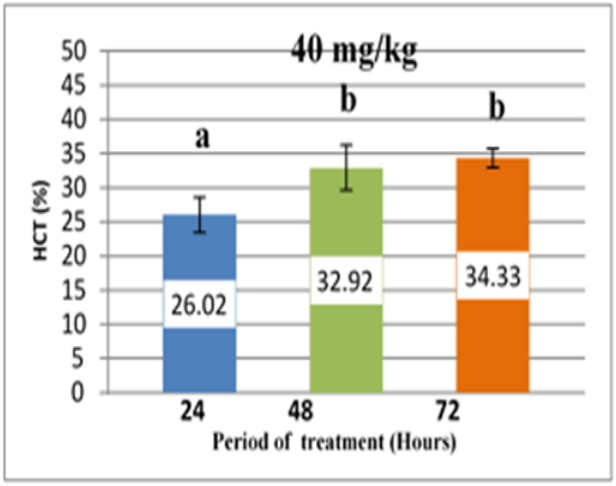


Figure (4-6A, B): HGB level in male rats treated with Isotretinoin (20 and 40) mg/kg,in different periods for 30 days, n=5. A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05





figures (4-7 A, B): HCT level in male rats treated with Isotretinoin (20 and 40) mg/kg in differentperiods for 30 days,n=5 B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05.

MCV results of Figures 4-8(A, B) showed that there were no significant ($P > 0.05$) differences for both treatments and for all time periods. The results of Figures 4-9(A, B) showed that there were no significant($P>0.05$) differences at the dose of 20 mg for all time periods, while the dose of 40 mg /kg showed that there was a significant increase ($P < 0.05$) at the time period 24 hours.

Figure 4- 10(A, B) for the MCHC showed showed a significant decrease ($P \leq 0.05$) in the dose of 20 mg/kg and in the time period of 48 hours, while it increased significantly ($P < 0.05$) in the dose of 40 mg/kg in the time period of 24 hrs.

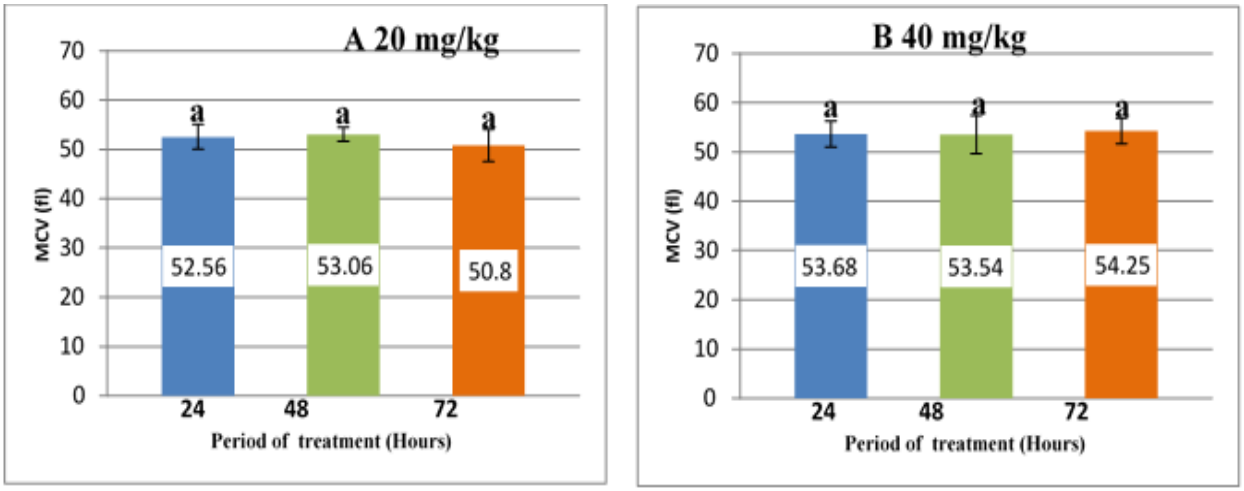


Figure (4- 8A, B): MCV level in male rats treated with Isotretinoin (20 and 40) mg/kgin different periods for 30 days, n=5. A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

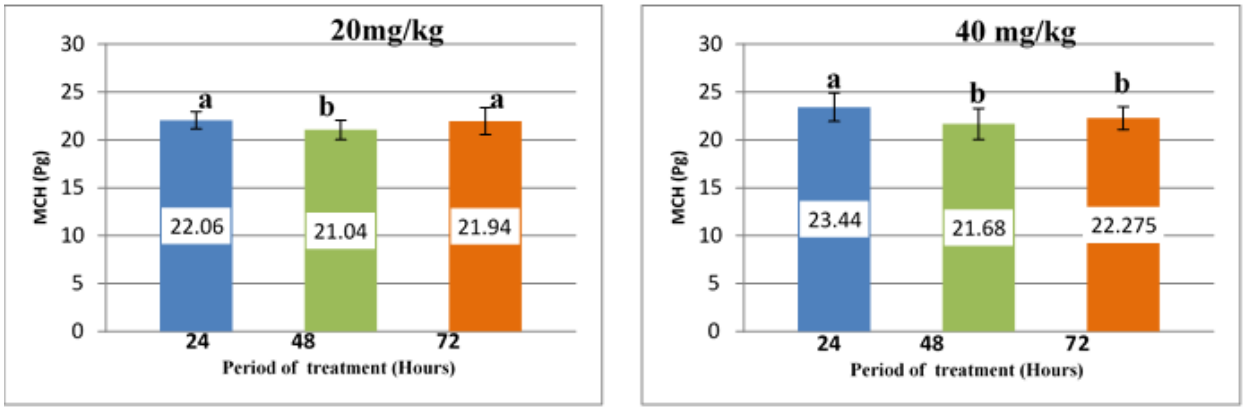


Figure 4-9(A, B): MCH level in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5. A(20mg/kg) ,B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

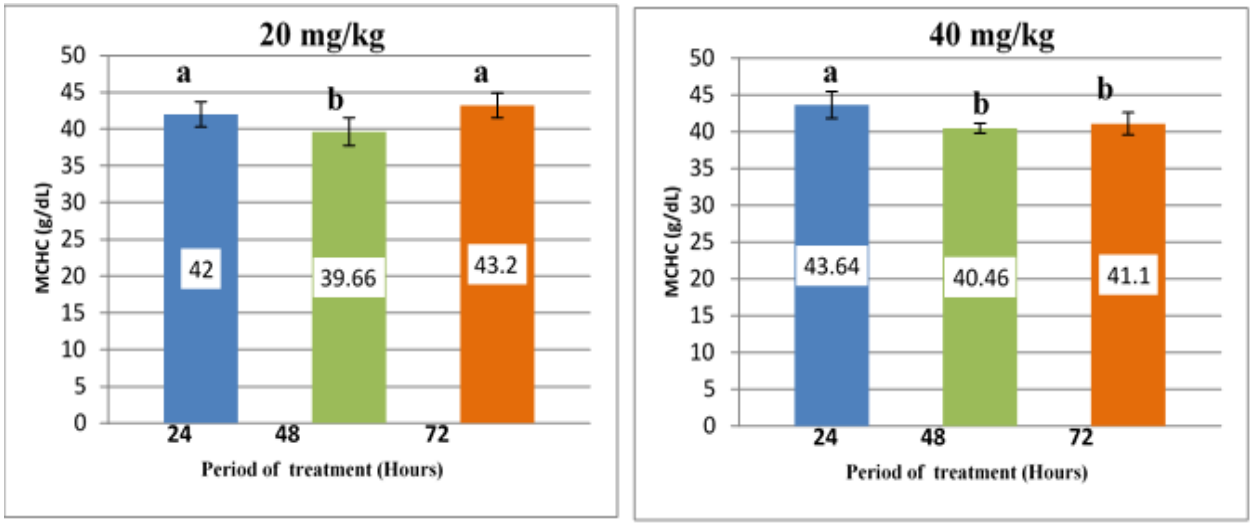


Figure 4-10(A, B): MCHC level in male rats treated with Isotretinoin (20 and 40) mg/kg in different period for 30 days,n=5. B (40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

The results of Figure (4- 11A, B) showed a significant increase (P<0.05) in the blood PLT at the time period 48 for the dose 20 mg/kg However, there were no significant (P >0.05) differences at the 40 mg/kg dose for all time periods

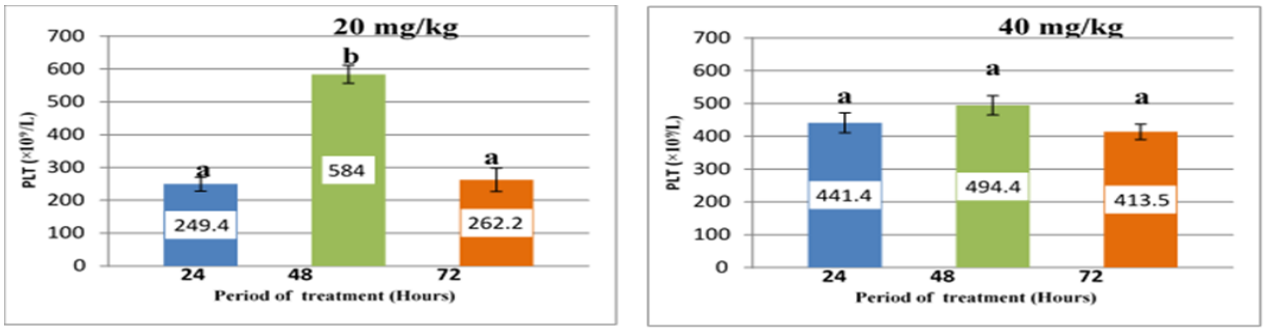


Figure 4-11(A, B): PLT level in male rats treated with Isotretinoin (20 and 40) mg/kg in different period for 30 days, n=5 A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

4.4 Effect of Isotretinoin Drug Does On Liver Functions:

The results of ALT showed a significant increase (P 0≤.05) at the dose of 20 mg for a period of 24 hours, and at of 40 mg/kg the significant increase (P ≤0.05) occurred in the time period of 24 and 72 hrs when compared with the control groups table (4-1).

The results of the AST table (4-1) showed that there was a significant increase (P ≤0.05) when treated with a dose of 40 mg for the time periods 24 and 72 hours, there were no significant (P>0.05) differences in the treatment with 20 mg/kg for all periods of time compared with the control groups in AST level.The results also clarified it was noted that there was a significant increase (P ≤0.05) at the dose of 20 mg/kg for all periods of time.

Table (4. 2): Liver Enzymes in males albino rates treated with Isotretinoin (20 and 40 mg/kg) in different periods for 30 days.

Groups / Parameters	Control (D.W)	Control (Oil)	20 (mg/Kg)			40(mg/Kg)			LSD (0.05)
			24h	48h	72h	24h	48h	72h	
	Mean±S.D								
ALT (IU/L)	41.00±1.6	43.40 ±5.8	65.40 ±10.2	47.00 ±6.0	46.60±16.5	71.25±7.4	53.60±3.7	57.80±5.5	**13.642**
AST (IU/L)	126.33± 11.1	126.20±36.3	235.60±11.4	114.20±21.3	142.40± 16.8	386.25± 29.1	260.40± 17.6	308.00±23.1	**167.889**
ALP(IU/L)	415.00± 10.8	454.66±24.1	810.66±21.0	607.80±14.4	609.20± 36.9	626.75± 17.3	599.20± 14.4	510.60±17.6	**247.871**

4.4.1 Effect of Duration of Isotretinoin Therapy on Some Liver Enzymes:

The results of Figure (4-12 A,B) showed a significant increase in ALT level at the dose of 20 and 40 mg at the time period of 24 hours compared to the time periods of 48 and 72 hrs.

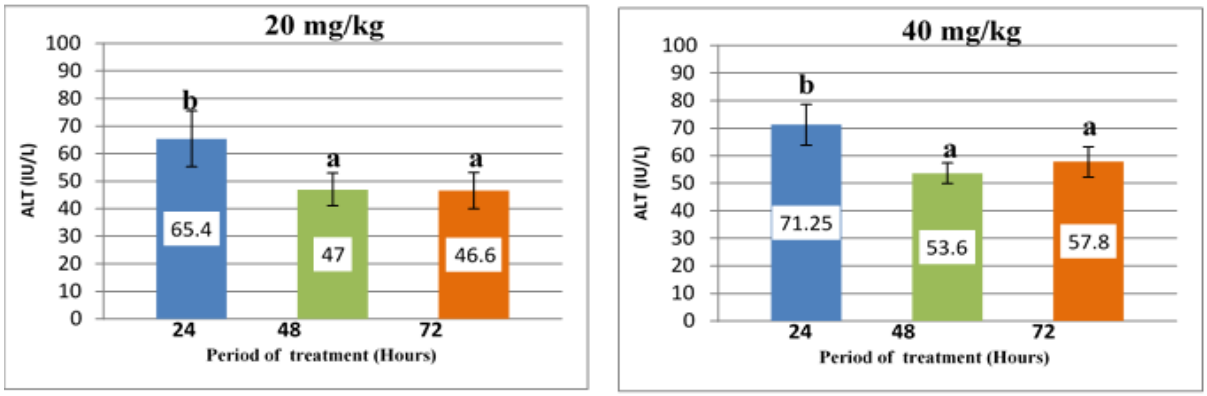


Figure 4-12 (A, B): ALT level in male rats treated with Isotretinoin (20 and 40) mg/kg in different period for 30 days, n=5. A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

The results of Table 4- 13(A,B) showed a significant increase ($p \leq 0.05$) in AST (IU/L) level in male rats treated with20 mg/kg for the time period 24 hours compared to the time periods 48 and 72 hrs.

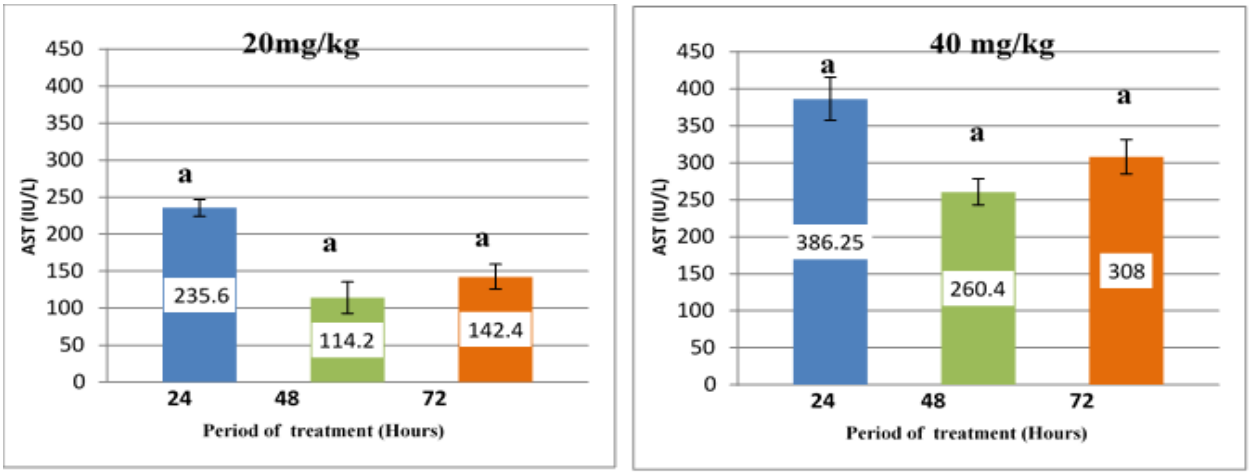


Figure 4-13(A, B): AST level in male rats treated with Isotretinoin (20 and 40mg/kg, in different period for 30 days, n=5. A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

The ALP results showed that there were no significant (P > 0.05) differences for both treatments and for all time periods (Fig.4-14 A, B).

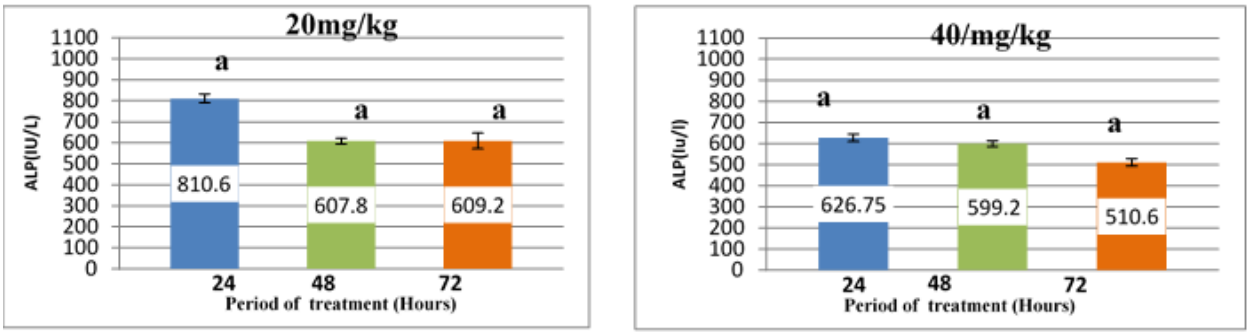


Figure 4-14(A, B): ALP level in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5. n=5. A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

4.5 Effect of Isotretinoin Does on Liver Damage Biomarker

The results in (table 4.3) showed that there was a significant increase (P ≤0.05) in FAPB 1 in male rats treated with 20 mg/kg at a period 48 and 72 hrs compared to the control groups. While the results of the kallistaien table (4-3) showed that there was a significant decrease (P ≤ 0.05) when treated with 40 mg/kg for a period of 24 hrs.

Table (4.3): Level of Liver damage biomarkers in male albino rats treated with Iso (20 and 40) mg/kg in different periods for 30 days.

Groups / Parameters	Control (D.W)	Control (Oil)	20 (mg/Kg)			40 (mg/Kg)			LSD (0.05)
			24h	48h	72h	24h	48h	72h	
	Mean±S.D								
FAPB(ng/ml)	6.75±0.5	7.86±1.7	7.08±2.3	8.35±0.6	8.75±2.3	8.03±1.1	7.44±0.8	8.06 ±1.7	1.029
Kalstainen(ng/ml)	71.78±4.0	64.19 ±5.8	58.44 ±3.9	64.19 ±7.4	64.08±8.1	55.93±9.7	61.26±7.3	59.52±8.4	6.483

4.5.1 Effect of Duration of Isotretinoin Therapy on the Biomarker of Liver Damage:

The results of Figures 4- 15 (A, B) showed that when the groups were compared each other, depending on the time period for taking the treatment, there was significant decrease (P≤0.05) in FAPB1 when the treatment of 20 mg in the time

period 24 hours compared to the time periods 72 and 48 hours, as it showed that there are no significant (P>0.05) differences in the treatment 40 mg for all time periods. The results of Figures (4- 16 A, B) It showed kallistainen that there were no significant (P>0.05) differences for both doses and for each time periods.

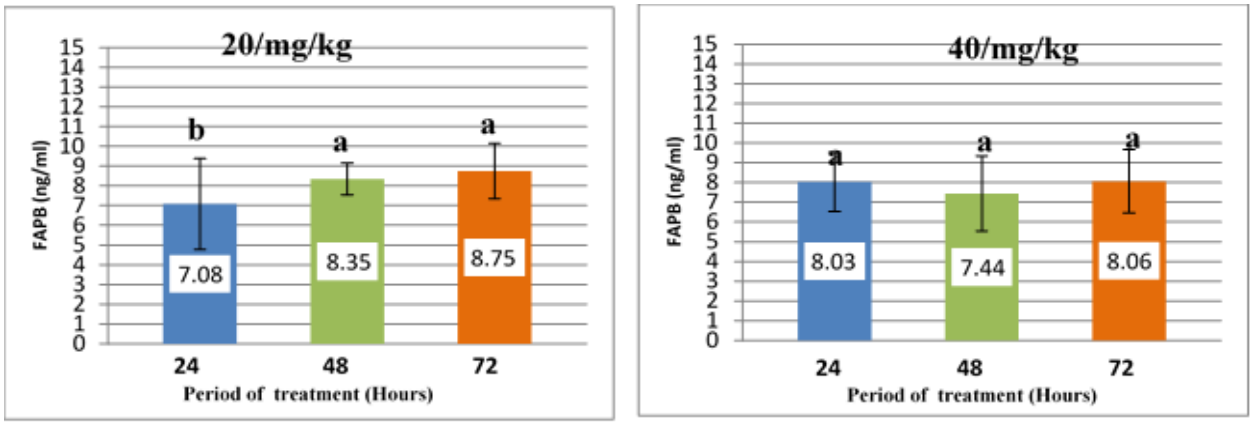


Figure 4-15(A, B): FAPB1 level in male rats treated with Isotretinoin (20 and 40) mg/kg,in different period for 30 days, n=5. A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

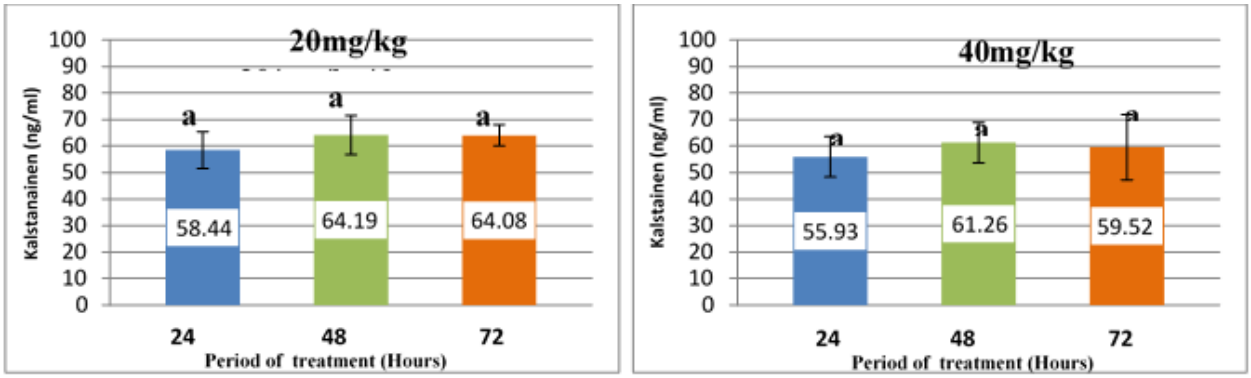


Figure 4-16(A, B): Kalstatainen level in male rats treated with Isotretinoin (20 and 40) mg/kg, in different period for 30 days, n=5. A(20), B(40 mg/kg) Different letters indicate a significant difference Similar letters mean there are no significant differences.LSD=0.05

4.6 Correlations Blood Parameters and SomeLiver Enzymes

The results showed that there was a significant negative correlation between WBCs and the percentage of lymphocytes (Fig. 4-17), and a positive significant correlation between WBCs and Mid% (Fig. 4-18). Also was a significant negative correlation between lymphocytes and mid%, as shown in Figures (4-19).

The figure (4-20) also showed a significant negative correlation between lymphocytes and granulocytes, it was also found that there is a positive significant difference between lymphocytes and MCHC, as shown in Figure (4-21), as in Figure (4-22) that there is a significant positive correlation between Mid and granulocytes. As shown in Figure (4-23) that there is a significant negative correlation between Mid and MCH and shown in Figure (4-24) that there is a significant negative correlation between Granulocytes and MCHC for doses of 40 and 20 mg and for all periods.

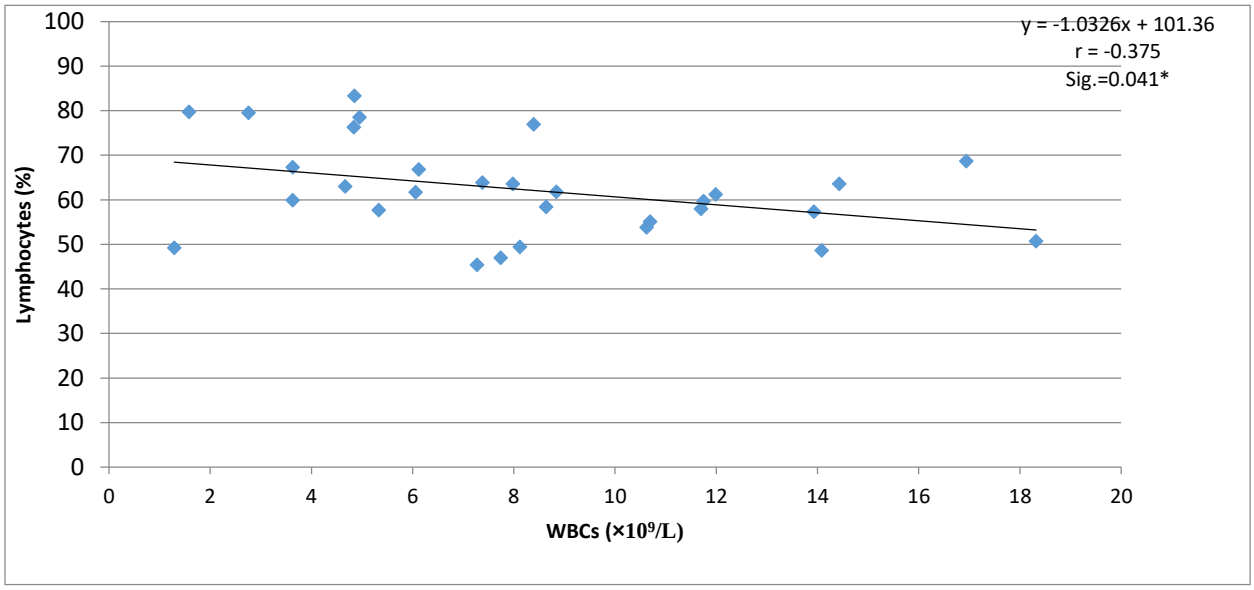


Figure (4-17): negative Correlation between WBCs and lymphocytes In male rats treated with Iso (20 and40 mg/kg/) in different period for 30 days n=5.

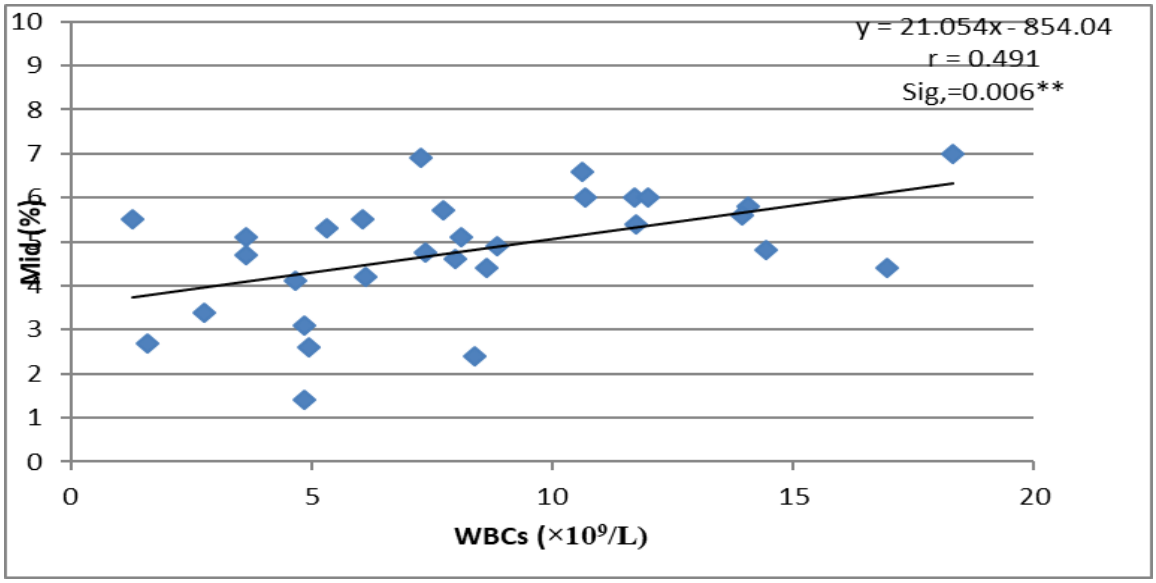


Figure (4-18): positive Correlation between WBCs and mid in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days n=5.

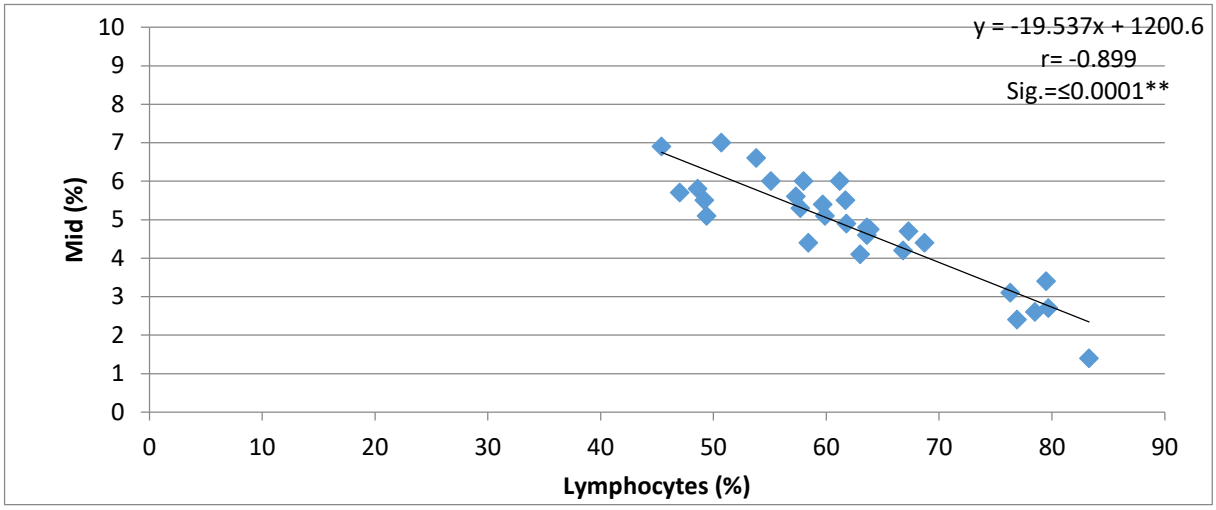


Figure (4-19): Negative Correlation between lymphocytes and Mid in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days.

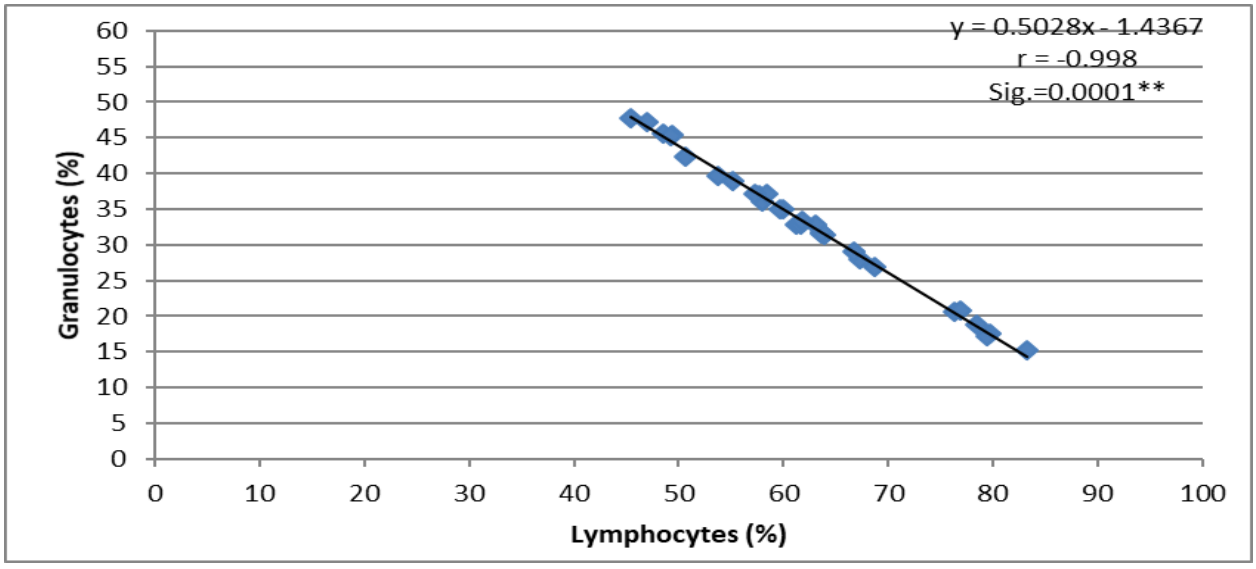


Figure (4-20): Negative Correlation between lymphocytes and granulocytes in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days

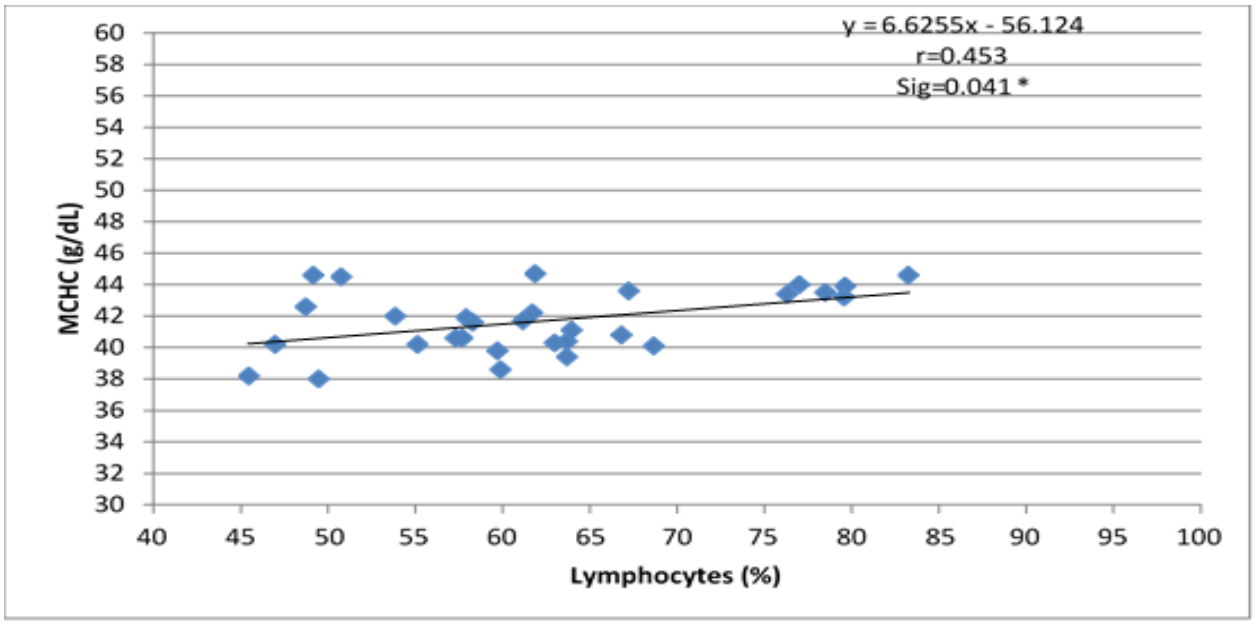


Figure (4-21): Correlation coefficient between lymphocytes and MCHC In male rats treated with Isotretinoin (20 and40 mg/kg/day) in different period for 30 days n=5.positive signification correlation

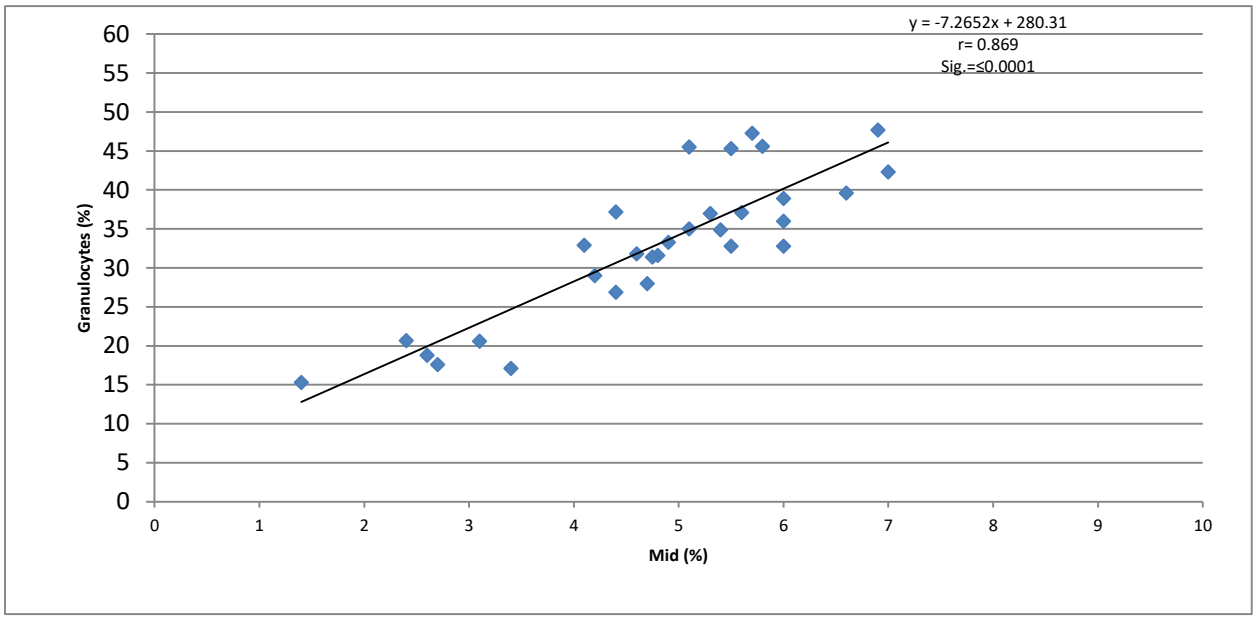


Figure (4-22): positive Correlation between Mid and Granulocytes in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days

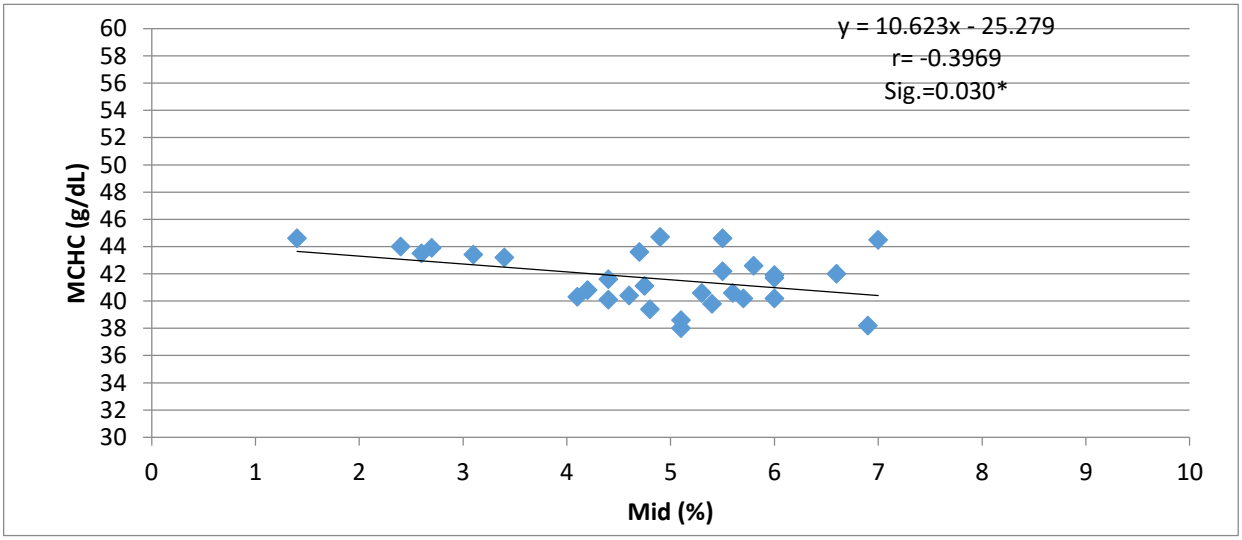
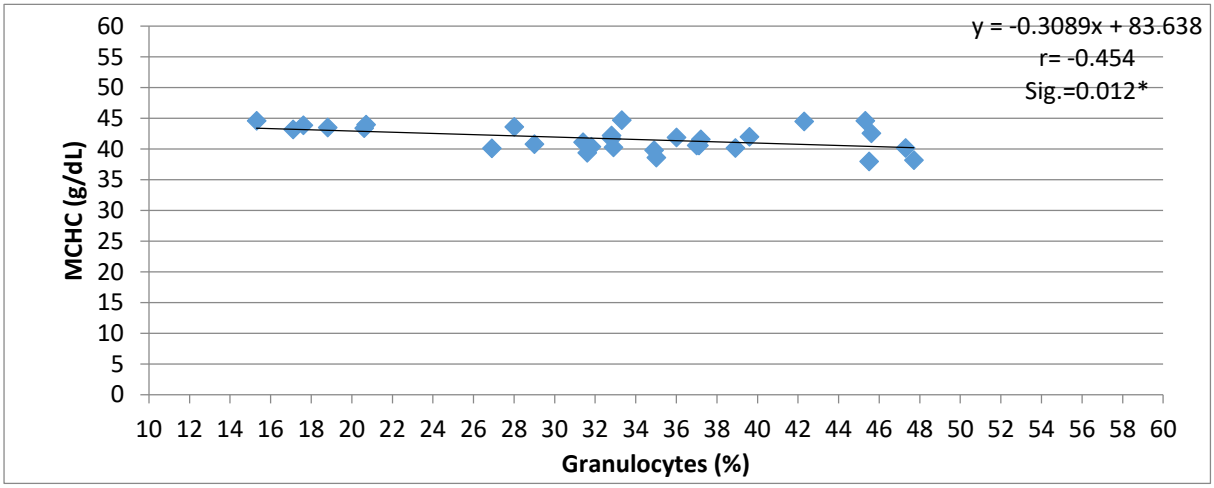


Figure (4-23): negative Correlation between Mid and MCHC in male rats treated with Isotretinoin (20 and40 mg/kg/day) in different period for 30days



Figure(4-24): negative Correlation between granulocytes and MCHC. in male rats treated with Isotretinoin (20 and40 mg/kg/day) in different period for 30 days

The Figure (4-25) showed that there is a significant positive correlation between both RBCs and HGB, as well as between RBCs and HCT as in Figure (4-26). As shown in Figure (4-27) there is a significant negative correlation between RBCs and MCHC as well as between RBCs and ALT in Figure (4-28). While figures (4-29) and (4-30) that there is a significant negative correlation between RBCs, AST and HGB, ALT. and shown in Figures (4-31) and (4-32), there is a significant negative correlation between HCT, ALT and HCT, AST.

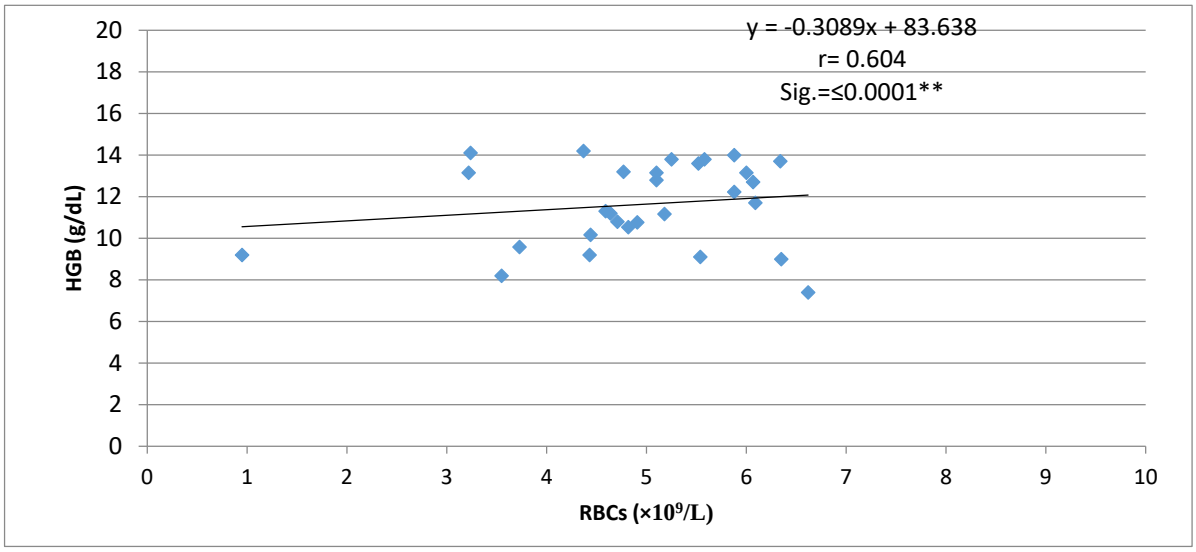
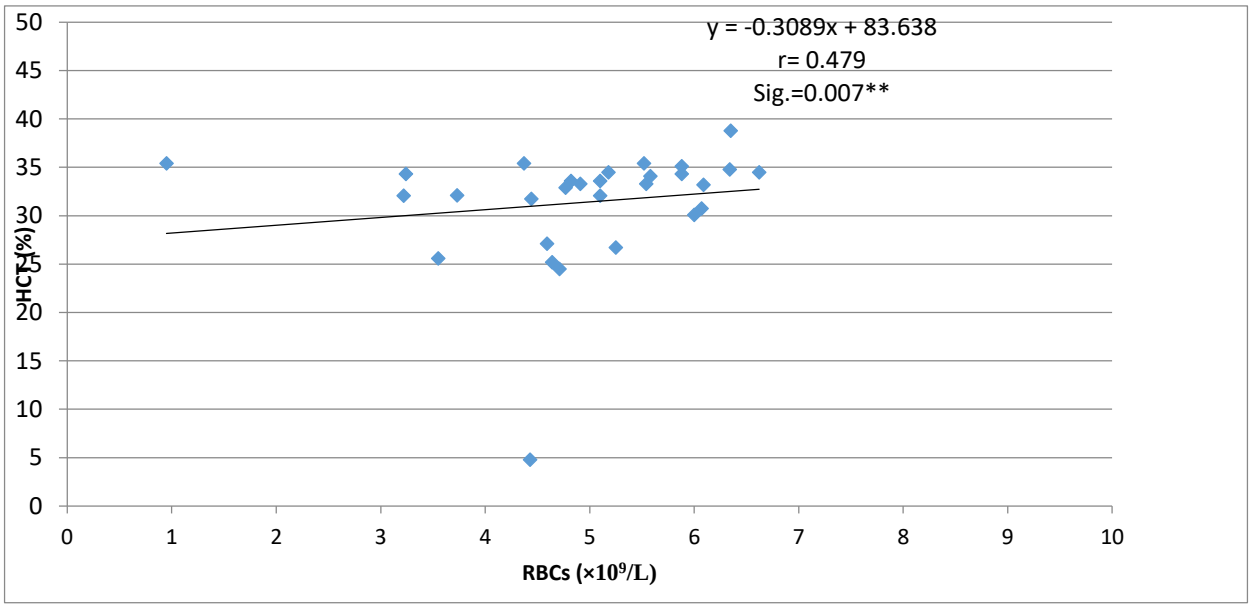


Figure (4-25): positive Correlation between RBCs and HGB. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days



Figue(4-26) positive Correlation between RBCs and HCT. in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days

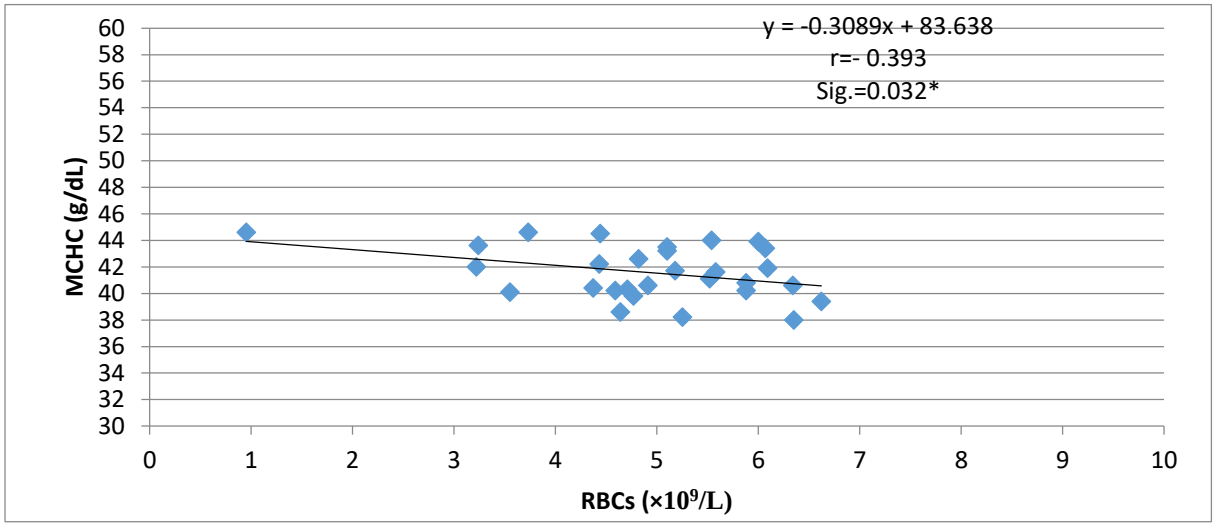


Figure (4-27): negative Correlation between RBCs and MCHC. in male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days

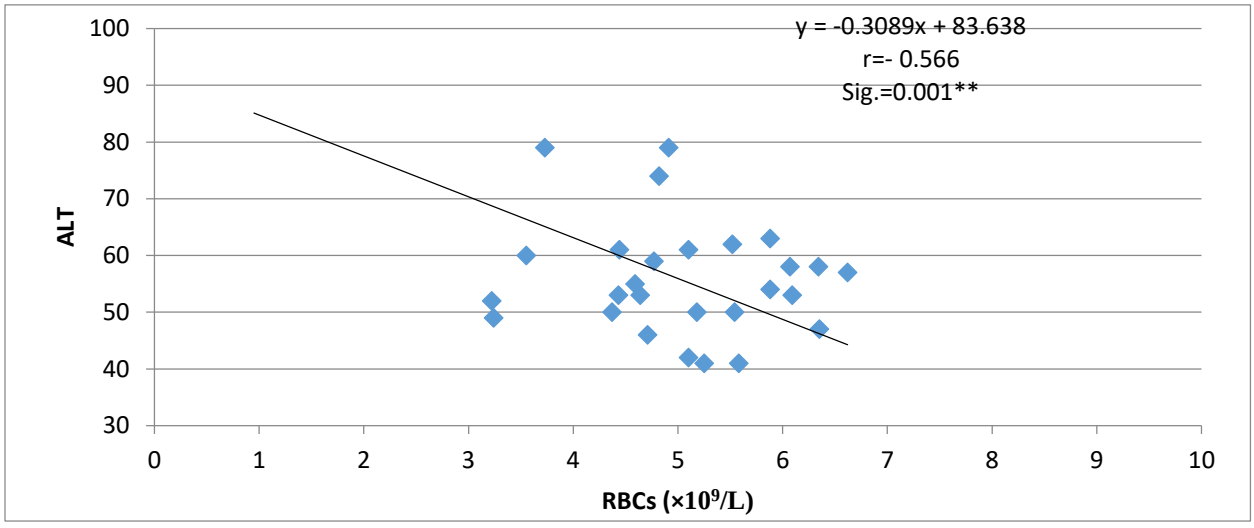


Figure (4-28) : negative significant Correlation coefficient between RBCs and ALT. In male rats treated with Isotretinoin (20 and40 mg/kg/day) in different period for 30 days correlation

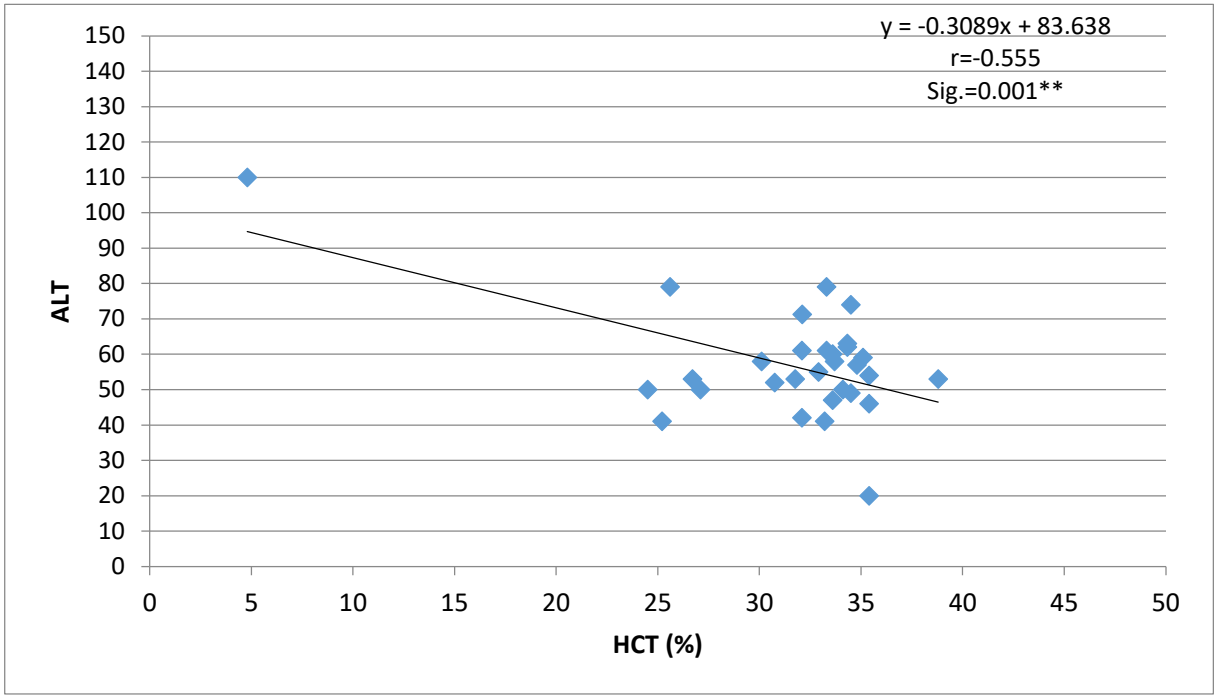


Figure (4-30): negative Correlation between HCT and ALT. in male rats treated with Isotretinoin 20 and40 mg/kg) in 30different period for 30

days n=5. negative significant correlation

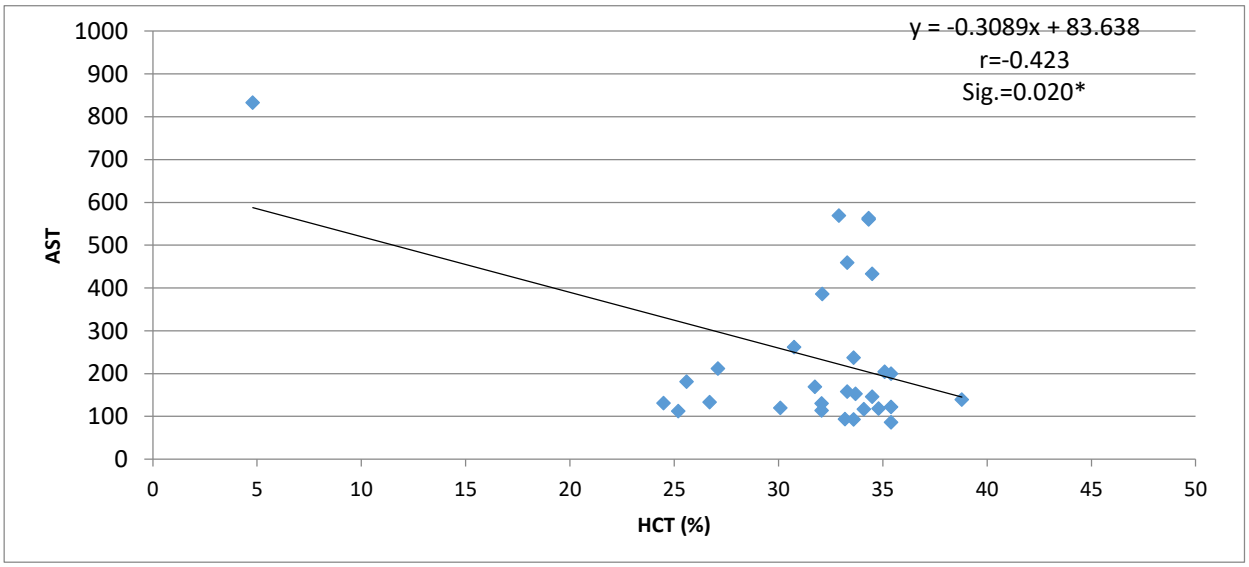
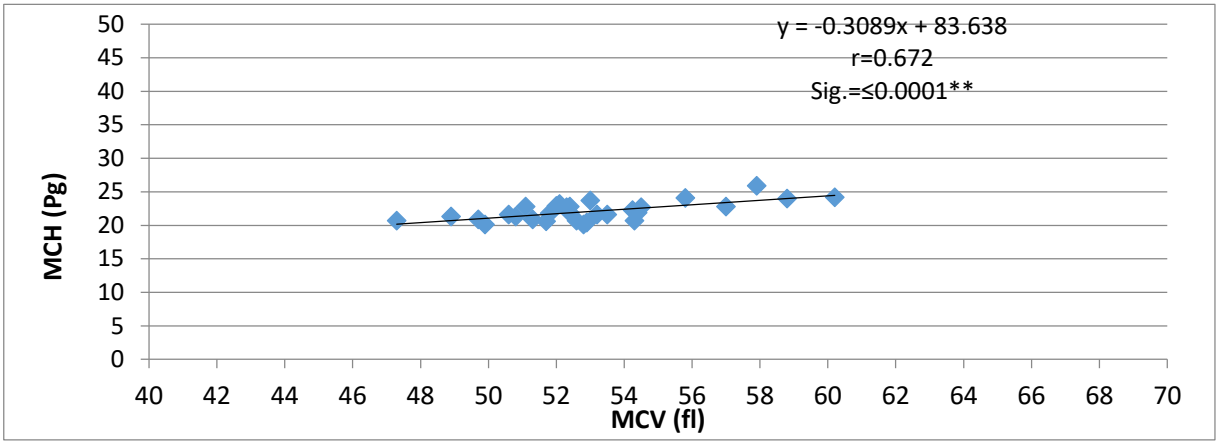
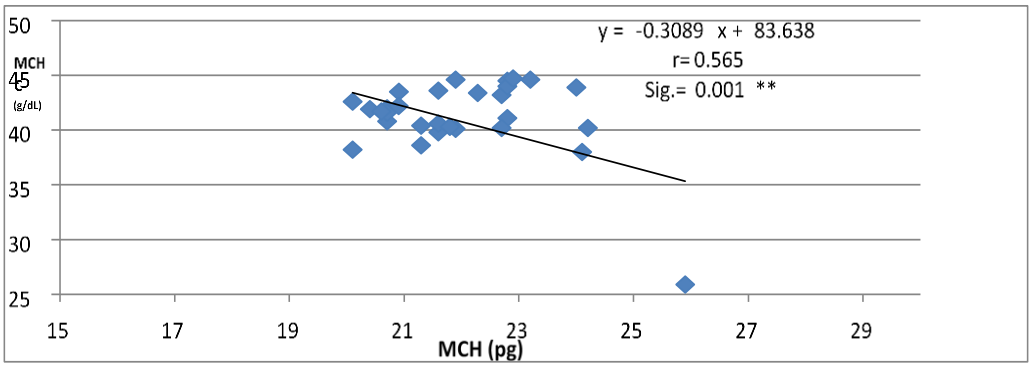


Figure (4-31): negative Correlation between HCT and AST. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days



Figure(4-32): positive Correlation between MCV and MCH. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days

It was observed from Figure (4-33) that there is a significant positive correlation between MCV and MCH for both doses and for all time periods. Figure (4-34) also showed that there was a significant negative correlation between MCH and MCHC for 20 and 40 mg and for all time periods.



Figure(4 -34): C orrelation between MCH and MCHC. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days n=5. a significant negative correlation

It was observed from Figure (4-35) that there was a significant positive correlation between ALT and AST for 20 and 40 mg and for all time periods.

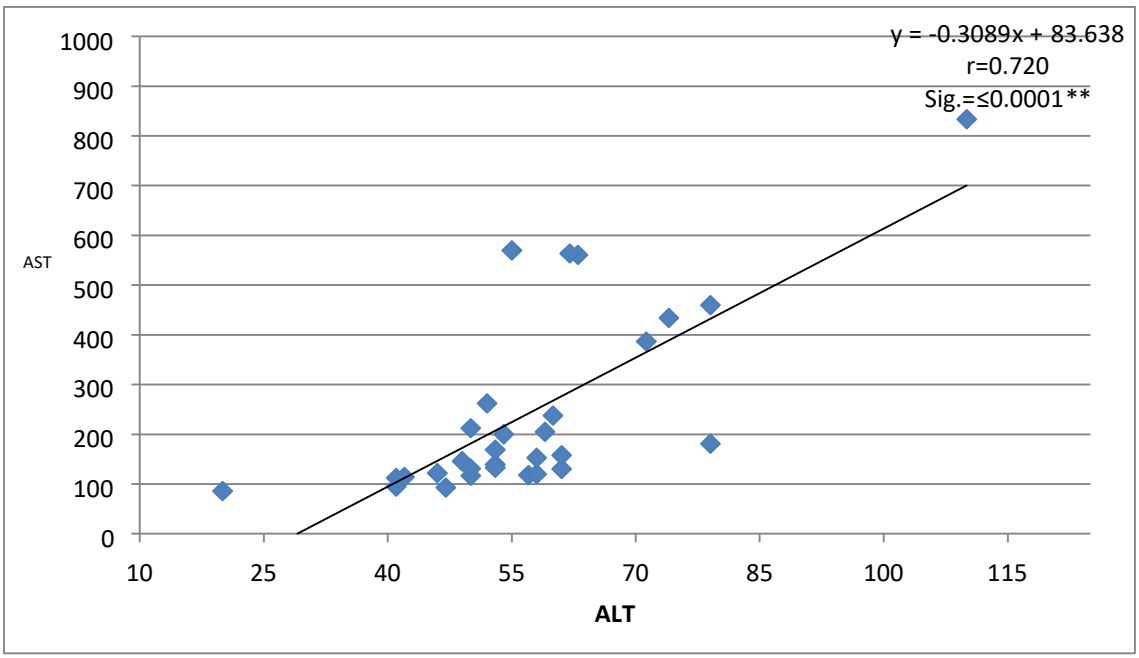


Figure (4 -35): Correlation between ALT and AST. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days n=5. significant positive correlation

The results of Figure (4-36) showed that there is a significant positive correlation between ALT and ALP for both doses and for all time periods. Figure) 4 -37) showed that there is a significant positive correlation between AST and dose concentration for all time periods.

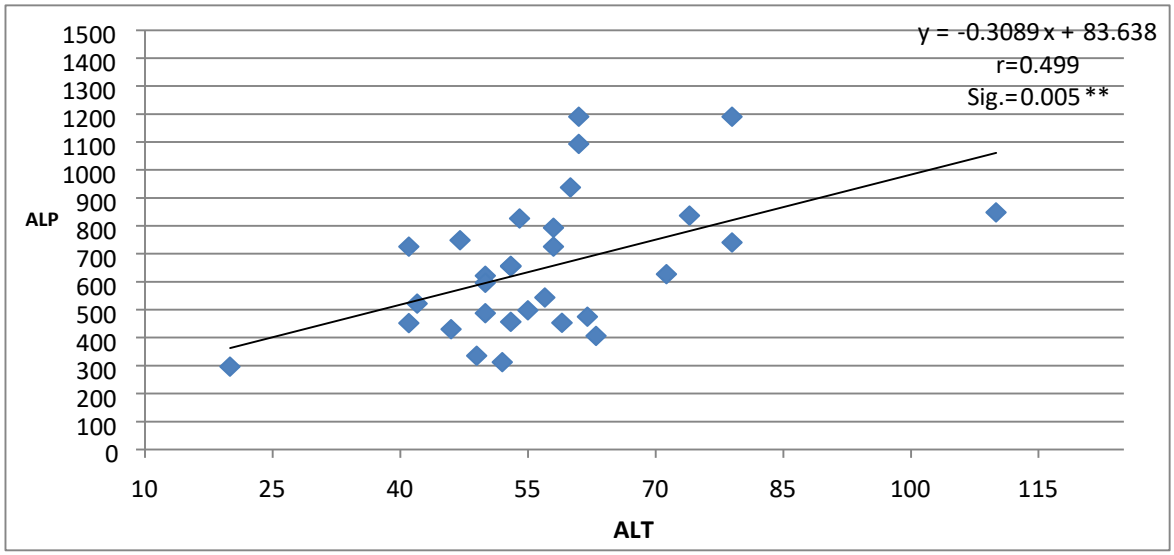


Figure (4-36): Correlation between ALT and ALP. In male rats treated with Isotretinoin (20 and40 mg/kg) in different period for 30 days n=5.signific positive correlation

4.7 Histopathological Study of Liver

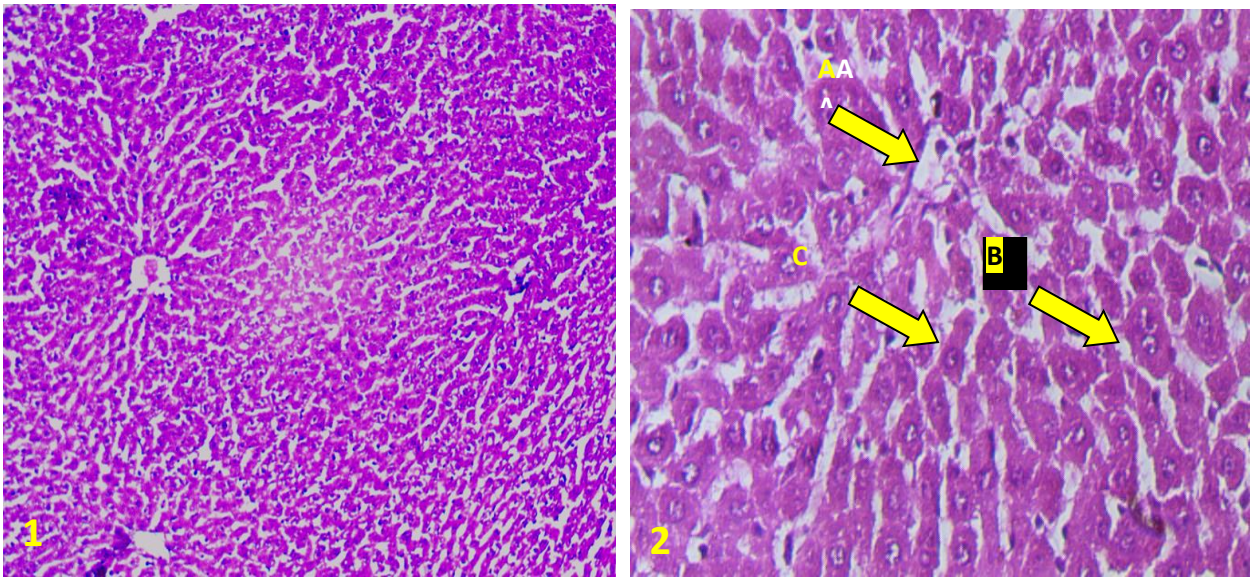
4.7.1 Effect of Isotretinoin (20 mg /kgs)

Photomicroscopes of rat's liver treated with(20mg/kg) for 24 hrs showed variable changes during a different of the study fig. (4-39) cross section rat liver showed treated with isotretinoin group 20mg/kg for 24 hrs normal hepatocytes while some bi-nucleated cells refer to regeneration.Photomicroscopes of rat's liver treated with(20mg/kg) for 48 hrs cross-section of the liver tissue of male rats is showed (4-40) as indicated by the presence of the central vein ,hepatocytes ,and sinusoids. Photomicroscopes of rat's liver treated with(20mg/kg) for72hrs cross-section of the Liver tissue of male rats is showed (4-41) central vein congested, K-cell proliferation, cellular swelling associated with hydropic degeneration.

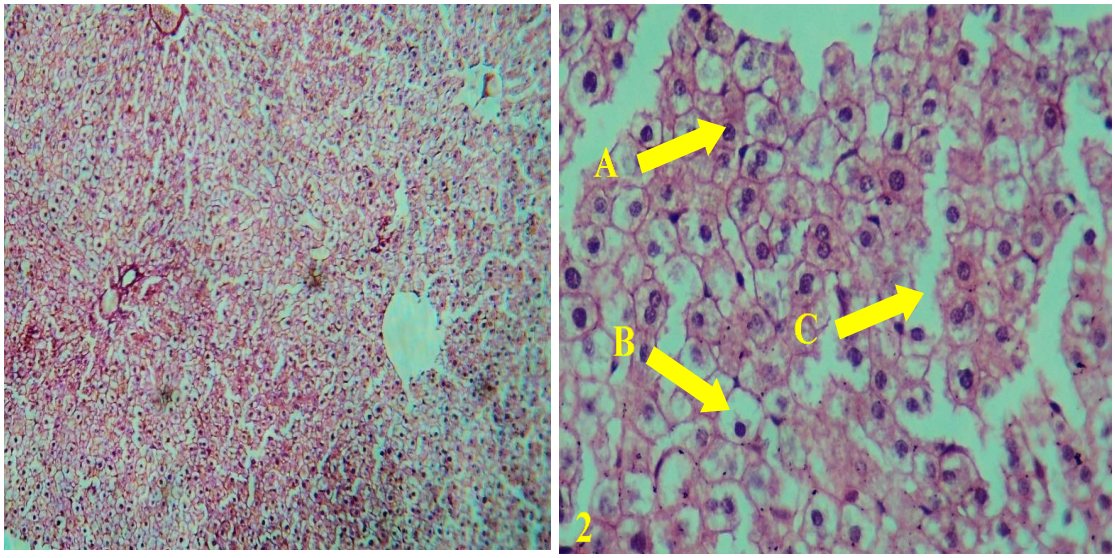
4.7.2 Effect of Isotretinoin (40 mg /kgs)

Photomicroscopes of rat's liver treated with (40mg/kg) for 24hrs cross section rat liver showed treated with isotretinoin (4-42) dilated central vein, dilated, congested sinusoids. Photomicroscopes of rat's liver treated with (40mg/kg) for 48 hrs cross section rat livershowed treated with isotretinoin (4-43) central vein congested, prominent K-cell, angiectasis. Photomicroscopes of rat's liver treated with (40mg/kg) for 72hrs cross section rat liver

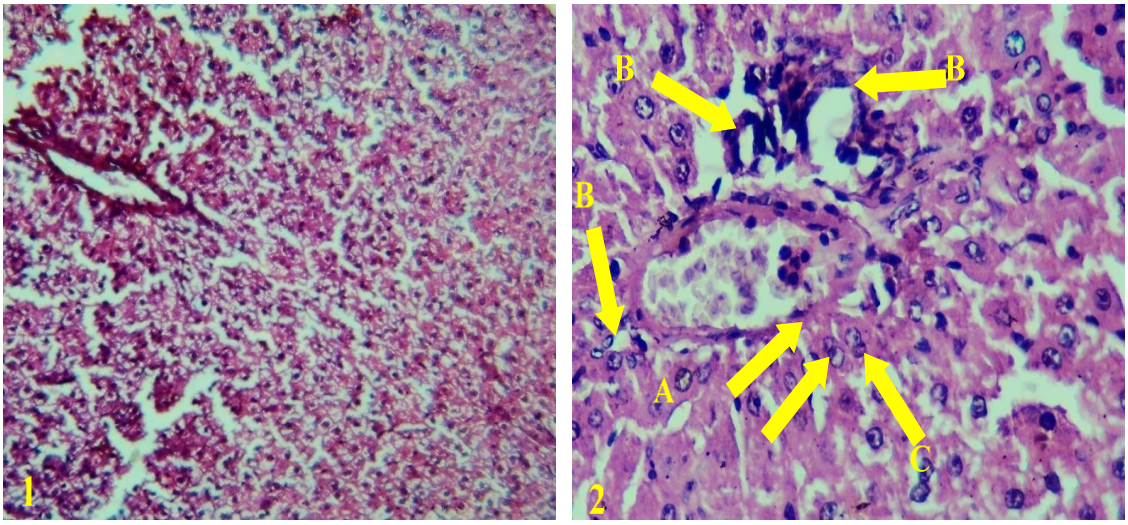
showed treated with isotretinoin (4-440) herniated central veins. These effects showed due to the administration of isotretinoin in different doses and durations compared to the group treated with oil fig. (4-38) show up marked fatty change and dilated sinusoids.



Figure(4-37)Cross section of a rat liver, showed the control group demonstrating normal hepatic architectures (10x) .(2) central vein (A), hepatocytes (**B**), and sinusoids (C) (H & E, 40x).



Figure(4-38). Cross section of rat liver showed treated with oil every day for 30 days, Cetral veins, Dilated sinusoids, marked fatty change (H&E,40x).



Figure(4-39)Cross section of rat liver showed treated with isotretinoin group 20mg,24 hours for 30 days

Dilated portal vein ,bile duct proliferation,(H&E,40X)

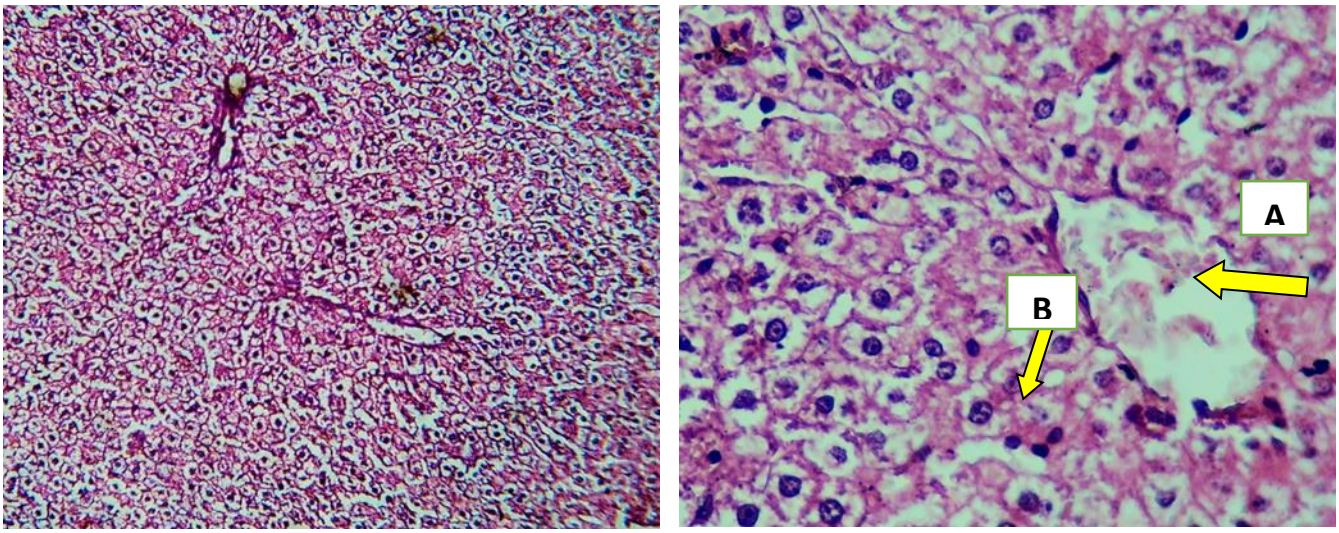
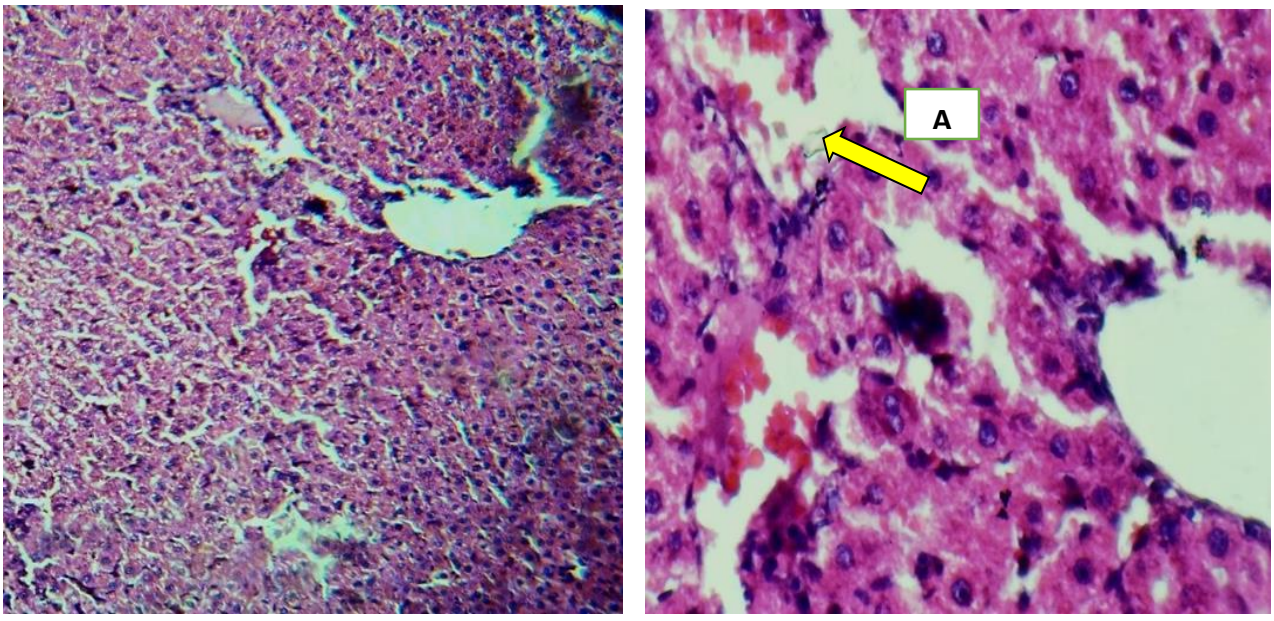


Figure (4-40) Cross section of the rat liver showed treated with isotretinoin group 20 mg/kg, for 72 hours for 30 days, central vein congested, Kupffer cell proliferation(A), cellular swellingassociated with hydropic degeneration(B) with karyolitic nucleus (D) (H & E, 40x).



Figure(4-41) Cross section of rat liver treated with isotretinoin 40 mg/kg ,24 hours for 30 days Central vein dilated , congested sinusoids sinusoids,

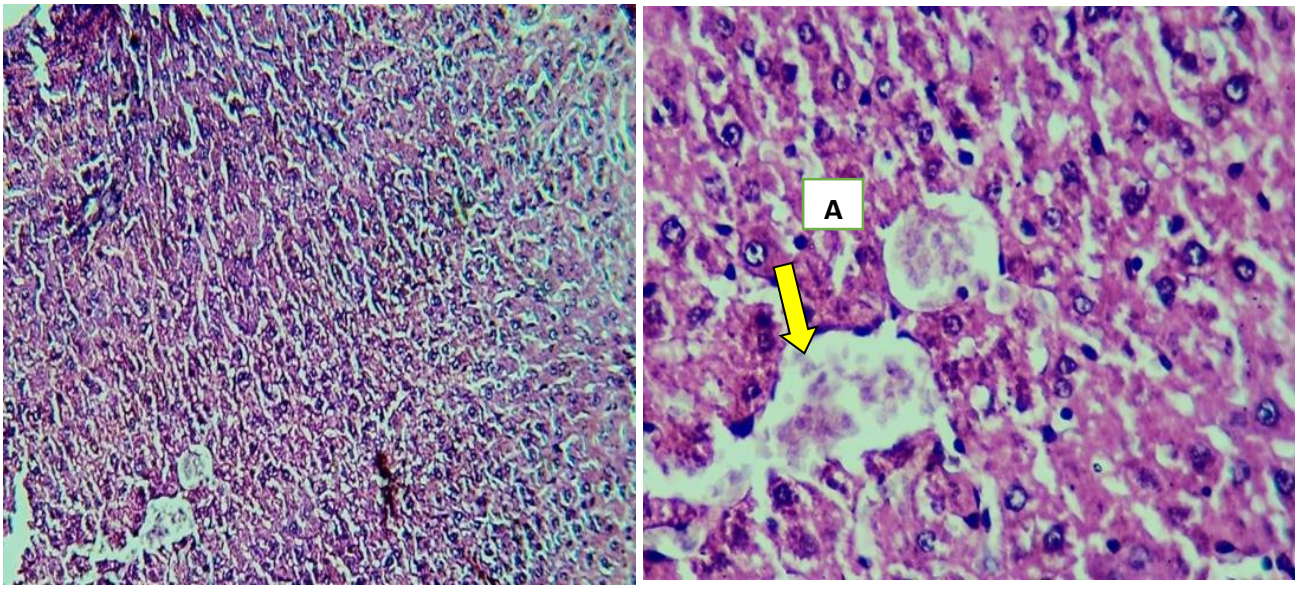


Figure (4-42) Cross section of rat liver showed treated with isotreinoin group 40mg ,48hour for 30 days Prominent k-cell,Angiectasis(A).(H&E40x).

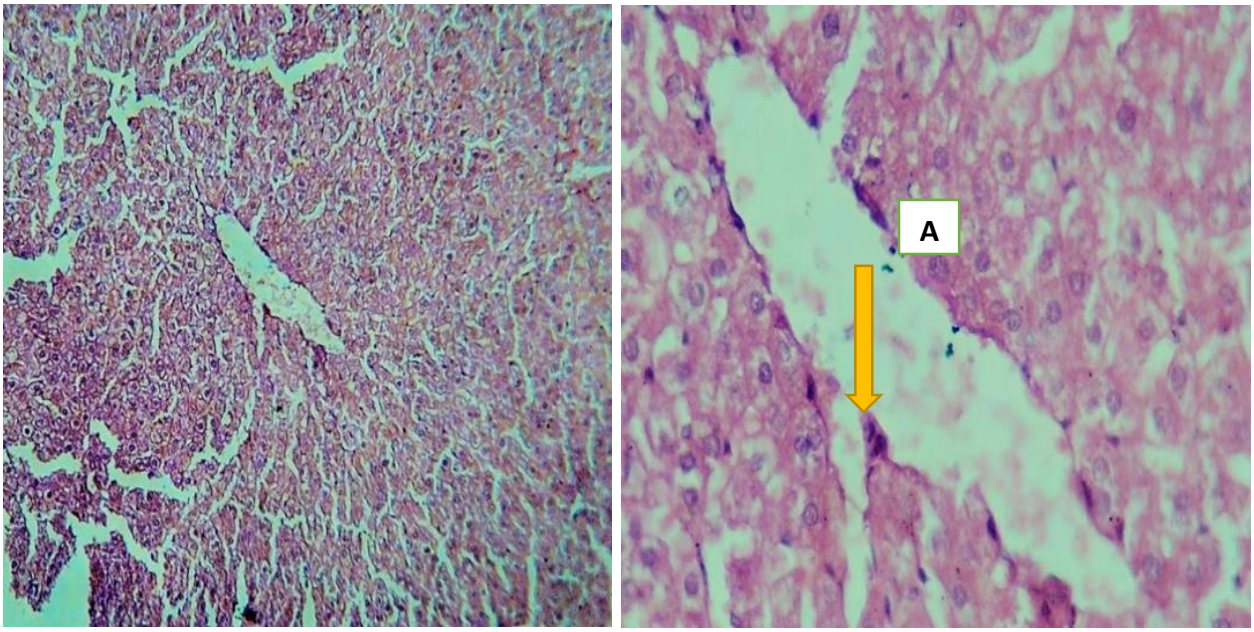


Figure (4-43) Cross section of the liver rat liver treated with isotretinoin 40 mg/kg, 72 hrs , marked pathological changes herniated centralveins (A) (H & E, 40x).

Chapter Five
Discussion

5. Discussion

5.1: Hematologyical parameter

The study by Karadag et al, (2013) when during treatment with ISO, treated rats found the HGB, HCT, and PLT parameters were found to be higher than in pre-treatment. A significant increase in the PLT parameter was also detected in while no difference was found in the HGB, HCT, and WBC parameters.

The result of the study of Karadag et al, (2013) did not agree with our study that found no change in these blood parameters (HGB, HCT, PLT). orally administered ISO to patients with acne vulgaris resulted in increased hematological parameters involving thrombocyte levels and decreased leukocyte and neutrophil levels. In the literature, ISO has been shown to have various and contradictory effects on platelet count and volume. For example, Karadag et al reported only a moderate increase in the platelet levels of 70 patients in their study. Any variation in the other hematological parameters (Hb, Hct, and WBC) in accordance with ISO therapy On the other hand, Bruno et al,1984 performed a study on 94 patients, and they did not detect any variation at all in the laboratory findings of patients using ISO therapy.

When hematological parameters were evaluated, the PLT, HCT, HGB, and LYM parameters were determined to be significantly higher during treatment compared to the pre-treatment levels (Douer et al.,2000). All Tralssrietineicaeid ATR, Seçkin et al, (2016) also reported that they found HGB and PLT parameters to be statistically significantly higher considering the fact that isotretinoin is also a vitamin A derivative, which can increase bone marrow with similar effects.

Few studies demonstrated isotretinoin-induced high PLT counts. The study of Karadag noted that the platelet count was modestly high following the isotretinoin treatment without any changes in the levels of WBC, Hb, and HCT. In another study, no sgnificant difference in HGB and HCT parameters was found, whereas the PLT

parameter was found to be significantly lower Thrombocytopenia may result from a bone marrow disorder such as leukemia or a problem with the immune system. Or it could result from the side effects of taking certain medications. It affects both children and adults. (Ataseven et al.,2014).

Gencoglan et al, (2018) it was revealed that PLT, WBC, and MCV parameters differed during treatment with ISO, and these changes appeared to fluctuate every month, but no significant difference was detected in other hematological parameters.

ISO has particularly played a role in causing thrombocytopenia, thrombocytosis, agranulocytosis, and leukopenia by means of affecting hematological parameters Thrombocytopenia may result from a bone marrow disorder such as leukemia or a problem with the immune system. Or it could result from the side effects of taking certain medications. It affects both children and adults. Thrombocytopenia can be mild and cause few signs or symptoms. In rare cases, the platelet count can be so low that dangerous internal bleeding occurs. Treatment options are available (Ertam et al.,2006).

Meanwhile, Schmutz et al., (2002) reported an increase in platelet levels caused by low doses of ISO.We found a modest rise in platelet count associated with ISO treatment. Several studies with other retinoids investigated platelet counts before and after treatment. All-trans retinoic acid (ATRA) stimulates megakaryopoiesis of progenitor cell line MEG-01 cells (Schweinfurth et al.,2010).

Yu et al, (2010) found that Retinoic acid when study the megakaryocytes viable for more than three weeks resulting the enhances the generation of hematopoietic progenitors from human embryonic stem cell-derived hematovascular precursors.

This effect does not appear to be of clinical importance in acne patients and our findings confirm that following blood counts is not necessary during ISO treatment

of acne. The mechanisms of ISO effect on hemoglobin parameters are not clear yet, In patients with decreased cell count levels, immune-mediated mechanisms, nonimmunological mechanisms, and bone marrow suppression were held responsible (Zane et al .,2006).

The reason for this may be look to Inflammation is an important factor in acne development. The anti-inflammatory and immune-modulatory effects of ISO have been well-documented. Karadag et al, (2012) reported statistically significant levels of pro-inflammatory cytokines, in conclusion, the detected decreased levels of NLR, an indicator of the inflammatory response, following treatment supported the prior research regarding the anti-inflammatory effect of ISO therapy in the treatment of acne vulgaris. This contradicts the results of our current study.

5.2 Liver enzyme:

Isotretinoin is still a very powerful treatment for moderate, severe, and recurrent acne, and it consistently results in dermatological improvement following therapy(Zaenglein et al.,2016).However, there are some side effects that limit the use of isotretinoin. Among the most worrying are the suspected teratogenic effects Studies confirm that longterm high-dose use of retinoid-containing drugs can cause liver dysfunction without liver damage. Hepatic dysfunction occurs in up to 15% of patients taking Iso (Kızılyel et al., 2014).

Several enzymes in liver tissue have been long considered effective biochemical markers to evaluate liver injury. The elevations of AST and ALT levels in the circulation indicate disintegration of the hepatic cell membrane of the liver moreover, prolonged destruction in the hepatic cells results in more cytoplasmic releases to exacerbate hepatic dysfunction with subsequent elevation of ALP (Plaa, 2010).

In the present work, the severe hepatic damage induced with ISO was evidenced by the elevation in liver enzymes (serum AST, ALT, and ALP). Consistence with these findings, Iso has been shown to increase serum. Animals exposed to ISO exhibited a considerable increase in thiobarbituric acid reactive material, nitric oxide, reduced glutathione, and the antioxidant enzyme activity of catalase and superoxide dismutase in liver tissue. The findings presented demonstrate unequivocally that Iso induces oxidative damage. aminotransferase activities and liver damage in patients (Brelsford and Beute, 2008; Erturan et al.,2012)

Brelsford et al,(2008) demonstrate that the use of oral isotretinoin for the treatment of acne caused elevations in serum triglyceride, ALT, and AST levels in the population examined in this investigation. These modifications did not necessitate stopping the course of therapy, but they may raise the patient's chance of developing liver and cardiovascular disease, supporting previously published research. To reduce the inherent hazards of this drug's administration, thorough monitoring with measures of lipid profiles and liver function tests is essential This rise may be caused by often indicating inflammation or damage to cells in the liver. Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes, into the bloodstream, elevating liver enzymes on blood tests.

Baxter et al,(2003) additionally, it's critical to stress the pharmacist's involvement in the pharmacotherapeutic follow-up interview to make sure that the medication is administered properly and that the patients are being properly monitored. ALT and AST are enzymes expressed in the liver, and their serum activities indicate the degree of liver tissue damage.In athletes, however, these enzymes may originate from different tissues; while ALT comes mainly from the liver, AST can be linked to muscle cell leakage (Banfi et al. 2012).

A statistically significant rise in liver enzymes was increased seen following isotretinoin administration in a study by Schulpis and a difficult-to-reduce rise in alanine amino transaminase (ALT) was seen after 20 weeks of isotretinoin treatment in a study by (Nazarian et al 2019). Isotretinoin did not raise liver enzyme levels in people with severe acne, according to a recent study by (Pona et al.,2021).

increases in serum levels of liver enzymes after treatment were observed in 2.09% of patients. reported that total cholesterol, serum triglycerides, and liver enzymes returned to normal limits within 2 to 3 weeks after cessation of isotretinoin (Brzezinski et al.,2017).

Waisman,(1988) reported a 16-year-old male patient with agranulocytosis and elevated levels of AST and ALT as a result of isotretinoin therapy. The granulocyte count reached normal levels 4 weeks after the drug was discontinued (Waisman., 1988).

A study in Egypt (2015) showed that serum cholesterol increased in the majority of patients during treatment and the ALT and AST levels were normal in all patients before treatment and significantly increased in the majority of patients during treatment (Ahmad.,2015).

In Brazil (2012), a retrospective study conducted through a record review of data of 70 patients showed that after 3 months of treatment with isotretinoin, AST and ALT levels increased in 8.6% and in 7.3% of the acne patients respectively (Vieira et al .,2012).

Entezari-Maleki, (2011) showed that SAST and SALT levels increased in 7% and 7.9% of the patients respectively after 3 months of treatment this may beliver enzymes might be discovered during routine blood testing. In most cases, liver enzyme levels are only mildly and temporarily elevated. Potentially indicating liver

damage, high liver enzymes are detected by a simple blood testn (2014), a retrospective study conducted among 322 medical records of patients in Trkey found that after 3-months of treatment, the levels of AST and ALT increased by 0.9% and 3.4% of the patients respectively All of these studies agreed with our study, where liver enzymes increased. Numerous investigations have found that isotretinoin treatment causes transaminase activity to significantly increase from baseline values by up to five times (Kızılyel et al,2014).

The incidence of abnormal elevation in ALT and AST in patients with acne treated with isotretinoin is low and was not associated with significant morbidity. Total cholesterol and triglycerides levels increased during the treatment and this was statistically significant but the increase in levels was mild. We found that patients with higher body weight are at higher risk of elevations in ALT and triglycerides levels at the end of the treatment course.

ALP is often used to assess the integrity of the plasma membrane (Akanji et al.,1993). Such that in tissue and serum this indicates possible damage to the outer cell border (plasma membrane) an increased level of ALP may lead to potential membrane damage because ALP is a membrane-bound enzyme (Ruothalo, 2008).

High levels of serum ALP activity are usually not observed in liver damage, cancer, and heart infections (Jaroslaw et al.,2009). Many dermatologists request monthly laboratory monitoring for their acne patients on isotretinoin (Shah, and Kroshinsky.,2021).

While liver enzymes are usually found in the liver, damage to of important organ causes the enzymes to leak into the bloodstream.This is consistent with our study, where the results obtained showed that there is an elevation in the level of liver enzymes. From the results of the study, it could be concluded that there was a

statistically significant difference in the level of liver enzymes before and after the use of isotretinoin (Zhou et al.,2022).

There was a weak positive correlation between the dose and/or the duration of taking the drug and the elevation in the liver enzymes the study revealed that the increased dose and duration of taking the drug for 4-6 months had a significant impact on the elevation of liver enzymes and cholesterol levels. It was recommended to conduct baseline laboratory investigations (liver enzymes and cholesterol) for all patients before starting treatment (Albhaisi et al.2022).

The dose and duration of treatment should be well monitored. Follow-up laboratory investigation should be done every month during the period of treatment.Although some serum lipid and hepatic enzyme levels in this study varied during isotretinoin treatment, they generally remained within the normal range and did not lead to a condition that would require discontinuation of treatment. Hematological parameters varied in the same way(Hoseini et al.,2022).

Some did not change and some were found to be high. However, since the mean ± standard deviation values remained within normal limits, there was no need to discontinue treatment, and no effect on the patient was seen. In light of all these findings, it can be said that oral isotretinoin treatment has a limited effect on hematological parameters, serum lipid levels, liver enzymes, and renal functions. Therefore, it is recommended that patients should be checked for laboratory tests less frequently if there is no hepatic or hematologic disease prior to treatment (Pastori et al.,2022).

5.3 Biomarker liver injury:

FABP1 Liver-type fatty acid-binding protein is mainly expressed in the liver as well as the proximal tubular epithelial cells in the kidney. In general, the proteins

and enzymes existing within the hepatocytes have the potential to become biomarkers,for instance, alanine aminotransferase, which reflects hepatocellular damage Hepatocytes generate L-FABP, a critical regulator of fatty acid metabolism, in a healthy liver environment (Kaikaus et al.,1993).

Hepatic damage is becoming more linked to the evolution of minor differences, implying that a rise in hepatic L-FABP expression is followed by a rise in serum L-FABP levels. Because ALT has a large molecular size of 96 KDa and a lengthy half-life, it may not be sensitive enough to detect tiny variations in hepatocellular damage.L-FABP levels have been linked to an increased risk of organ failure (Karvellas et al.,2021).

L-FABP levels in the blood are now widely accepted as a reliable biomarker for detecting drug-induced liver impairment (Church et al.,2019). Furthermore, serum L-FABP levels will be valuable in predicting survival rates in individuals with acute and chronic liver illness. When compared to results obtained from patients without HCC, serum L-FABP levels were observed to be enhanced in the presence of HCC other than L-FABP up-regulation mechanisms may exist in the environment linked with HCC development, implying that other LFABP up-regulation processes exist. Serum L-FABP might be used instead of aminotransferases as a more sensible option for detecting tiny variations in drug treatment responsiveness trials. This is consistent with the results obtained through conducting the experiment. It was observed that FABP1 increased significantly after exposure to rats, and the effect of treatment on the liver caused tissue damage. Through these studies, we conclude that the higher this criterion, the higher the probability of tissue damage.

Kallistatin(kallikrein-binding protein) is a newly discovered serine protease inhibitor that is generated and expressed mostly in the liver and transported throughout the heart, kidneys, and blood vessels (Chao et al.,2018).

Kallistatin is a protein that is largely generated and released in the liver. Eyes, kidneys, liver, pancreas, heart, arteries and veins, atheroma, blood cells, and bodily fluids all release it in low concentrations. It has anti-tumor growth, anti-inflammatory, anti-oxidant, anti-angiogenic, and anti-inflammatory properties Because it is a negative acute-phase protein, its blood level drops as liver function declines. As a result, serum kallistatin levels might be used as a biomarker for cirrhosis and fibrosis of the liver (Chao et al.,2006).

Wu et al, (2019) this protein have anti-inflammatory, antioxidant, antiangiogenesis, and anti-tumor properties.Kallistatin has been shown in several trials to be an excellent biomarker for the early identification of liver fibrosis in a variety of liver disorders (Abas et al.,2019).

According to many research, kallistatin concentrations change across chronic liver disorders, which might be linked to impaired hepatic protein secretion activity (Nallagangula et al.,2017).

Cheng, et al (2015) Kallistatin levels were found to be considerably reduced in patients with liver fibrosis It was demonstrated that even a single biomarker level assessment may diagnose patients in the early stages of liver fibrosis with a sensitivity of 96.7 percent and a specificity of 50%. These findings back with our findings, which showed that kallistatin levels differed between patients with and without fibrosis.

Prystupa et al (2019) Blood kallistatin levels diminish as liver parenchymal damage develops in individuals with alcoholic liver cirrhosis, and compensated cirrhosis patients showed higher kallistatin activity than decompensated cirrhosis patients. Our data also suggest that kallistatin levels decrease with the severity of liver fibrosis. This is due to kallistatin's antifibrotic properties According to the research, an increase in liver fat might lead to a reduction in kallistatin production

as a result of lipopolysaccharide inflammation. It might indicate that kallistatin protects the liver parenchyma against pathogenic alterations (Li et al.,2019).

The inverse associations found between kallistatin and ALT, AST, and CRP show that persistent systemic and local inflammatory processes diminish the antiinflammatory marker's activity. However, literature evidence suggests that kallistatin has a modest sensitivity (64%) and specificity (77%) as a marker of liver cirrhosis. The kallistatin determination sensitivity was somewhat greater in the research of Halla et al (2017) in the identification of liver fibrotic alterations, at 96.7 percent, while the test's specificity dropped to 50%. The sensitivity and specificity of kallistatin testing were increased to 90 and 76.8%, respectively, when non-invasive scales for liver fibrosis and steatosis evaluation were used together. It is adequate for kallistatin determination as a biomarker for the early identification of liver fibrosis. This shows the agreement of the results of this study with previous studies, meaning that the lower the value of this criterion, the greater the damage to the liver.

5.4 Histological Study

The liver is the largest solid organ in the body and consists of parenchymal cells (hepatocytes) and many different types of nonparenchymal cells, such as hepatic stellate cells (HSCs), Kupffer cells (KC), and liver sinusoidal endothelial cells (LSECs) (Eguchi and Iwasa.,2021).

Hepatocytes are the most abundant cell type in the liver and they play an important role in basic organ function, including lipid and glucose metabolism, detoxification, and coagulation, with immune response under the purview of nonparenchymal cells. When the liver is in a disease state, parenchymal and nonparenchymal cells communicate with each other to accelerate liver damage;

hepatic damage triggers the production of cytokines, chemokines, and extracellular vesicles.The result of this study clearly shows the effect of isotretinoin on the rat's liver functional enzymes

The variances in the liver function enzymes during the different medicating duration and different dose of Iso was confirmed by many of the studies (Okan Kizilyel, et al .,2014).

Some of these studies indicate that the level of serum AST and ALT remain under the normal range or little increase during medication with a low dose (Ahmad , 2015), and that was agreed with the result of this study as itclearly saw no significant differences in the level of AST and ALT of the low dose isotretinoin (20mg) treatment group compared with the control group While a significant difference ($P<0.05$) was noticed in the level of ALT in the group which treated with a high dose of isotretinoin (40mg) as it appears that was agreed with the study of (Okan et al ., 2014).

The mechanism by which isotretinoin causes serum aminotransferase change is not known, but it may be described as a direct toxic effect that appears more frequently with higher dose therapy,The alkaline phosphatase level, is usually not affected by the isotretinoin dose because of that it’s ignored by most of the study. But in the study low dose of isotretinoin significant differences). The isotretinoin hepatotoxicity and liver injury (Saied and Hamza .,2014).

The histopathological changes of low and high doses of isotretinoinn described in this stud include these changes, Photomicroscopes of rat’s liver treated with(20mg/kg) for 24 hrs showed variable changes during a different of the study fig. (4-34) cross section rat liver showed treated with isotretinoin group 20mg/kg for 24 hrs normal hepatocytes while some bi-nucleated cells refer to regeneration (Byrne et al .,2022).

Photomicroscopes of rat's liver treated with(20mg/kg) for 48 hrs cross-section of the liver tissue of male rats is showed (4-35) as indicated by the presence of the central vein ,hepatocytes ,and sinusoids. Photomicroscopes of rat's liver treated with(20mg/kg) for72hrs cross-section of the Liver tissue of male rats is showed (4-36) central vein congested, K-cell proliferation, cellular swelling associated with hydropic degeneration (Deklotz *et al* .,2017).

Photomicroscopes of rat's liver treated with (40mg/kg) for 24hrs cross section rat liver showed treated with isotretinoin (4-37) dilated central vein, dilated, congested sinusoids. Photomicroscopes of rat's liver treated with (40mg/kg) for 48 hrs cross section rat livershowed treated with isotretinoin (4-38) central vein congested, prominent K-cell, angiectasis. Photomicroscopes of rat's liver treated with (40mg/kg) for 72hrs cross section rat liver(Okan Kizilyel, *et al* .,2014).

showed treated with isotretinoin (4-39) herniated central veins. These effects showed due to the administration of isotretinoin in different doses and durations compared to the group treated with oil fig. (4-33) show up marked fatty change and dilated sinusoids(Eguchi and Iwasa.,2021).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions and Recommendations

Conclusions

1- The drug had an effect on blood parameters and liver function and tissue the concentration of 40 mg/km was the most effective concentration.

2-The duration of administration of the drug every 72 hours showed the least effect in most of the criteria.

3- The drug had an effect on some biomarkers that indicate tissue damage, as the value of FABP1 increased in the serum of animals treated with isotretinoin and the value of kalstainen decreased in the serum of treated animals.

4-There was a clear effect on the liver tissue, as the pictures showed the presence of histological changes in most of the treated animals.

Recommendations

1- Studying the effect of the drug with the same duration on the kidney tissue .

2- A study of some modern biomarkers of the thyroid gland and its relationship to isotretinoin therapy.

3- Studying the effect of drug treatment on some genetic changes associated with the liver.

4- Studing the effect of drug on liver with high doses in rats.

5- Uses histochemistry stain to observe.

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APPENDIX

Table (3.1): Instruments and their suppliers (Appendix 1).

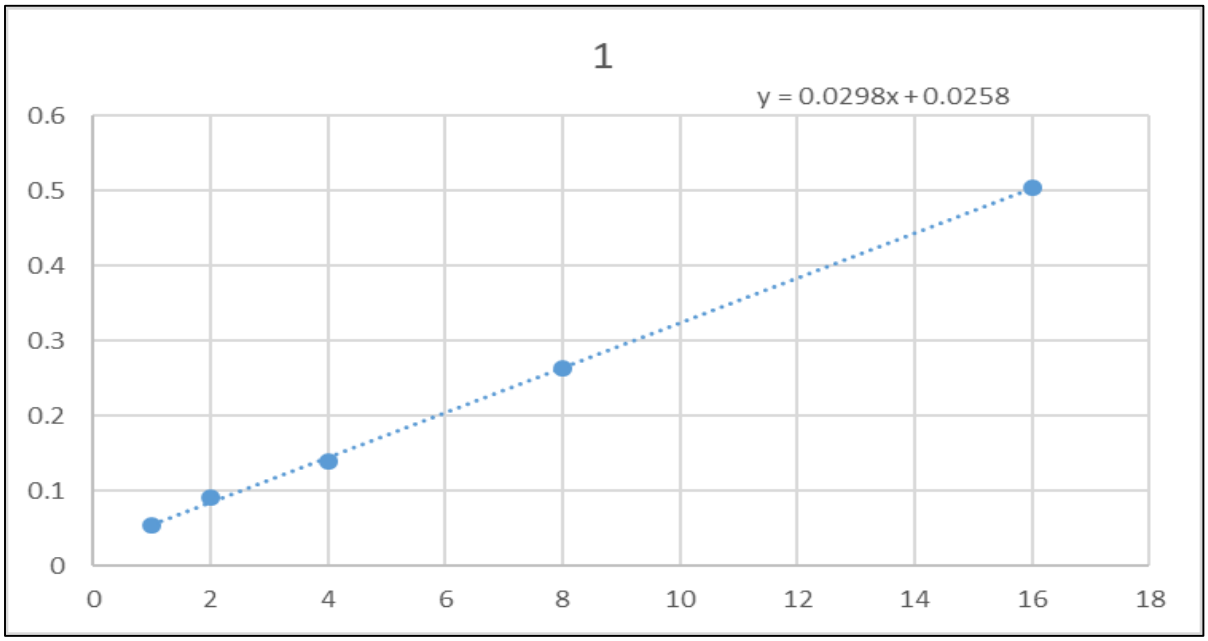
No.	**Equipment and Tools**	**Suppliers**
1	Centrifuge	Gemmy- Taiwan
2	Collection Tube	IBI Scientific- USA
3	Digital Camera	Genex- Germany
4	Disposable Tip	Indiamart- India
5	Dissecting Set	Elphor- Germany
6	Electrical Oven	Memmert- Germany
7	Electronic Micropipette	Slamed- Germany
8	Eppendorf Tube	Firatmed- Turkey
9	Hematological and analyzar	Lassco- India
10	Slide	Happy Science- China
11	Hot plate	Histo-Line- Italy
12	Latex Glove	HiGeen- Gordan
13	Light Microscope	Genex- USA
14	Medical Syringe	Shengguang- China
15	Microtome	RWD- China
16	Multi-Channel Dispenser Micropipette	Slamed- Germany
17	Refrigerator	Vestel- Turkey
18	Surgical Blade	Aspen Surgical- USA
19	Water Path	Raymond- England

Table (3.2): Chemicals, biologicals, and their manufacturers used in this study (Appendix2)

No.	Chemicals and Kits	Manufacturers
1	Absolute Ethanol	Scharlau- Spain
2	Albumin	Scharlau- Spain
3	Chloroform	BDH- England
4	D.P.X	BDH- England
5	Eosin	BDH- England
6	Formaldehyde (37%)	Merck- Germany
7	Hematoxylin	BDH- England
8	Hydrogen Peroxide (H2O2)	Sigma-Aldrich- Germany
9	Methanol (100%)	Scharlau- Spain
10	Paraffin Wax	GCC- England
11	Xylene	Scharlau- Spain
12	L-FABP ELISA kit	BT LAB , China
13	Kallistatin ELISA kit	BT LAB , China
14	AST kit	FUJIFILM-Japan
15	ALT kit	FUJIFILM-Japan
16	ALP kit	FUJIFILM-Japan
17	Oil	Turkie

3.2.4.1 Rat liver type fatty acid-binding protein ELISA kit Assay (Appendix 3)

L-FABP was evaluated from rats serum with commercially available ELISA kits, according to the manufacturer's instructions (BT LAB, China).



(3.2): standard curve of L- FABP

3.2.4.2Rat Kallistatin ELISA Kit (Appendix 4)

The value for kallistatin was estimated according to the manual procedure of the BT LAB kit (china).

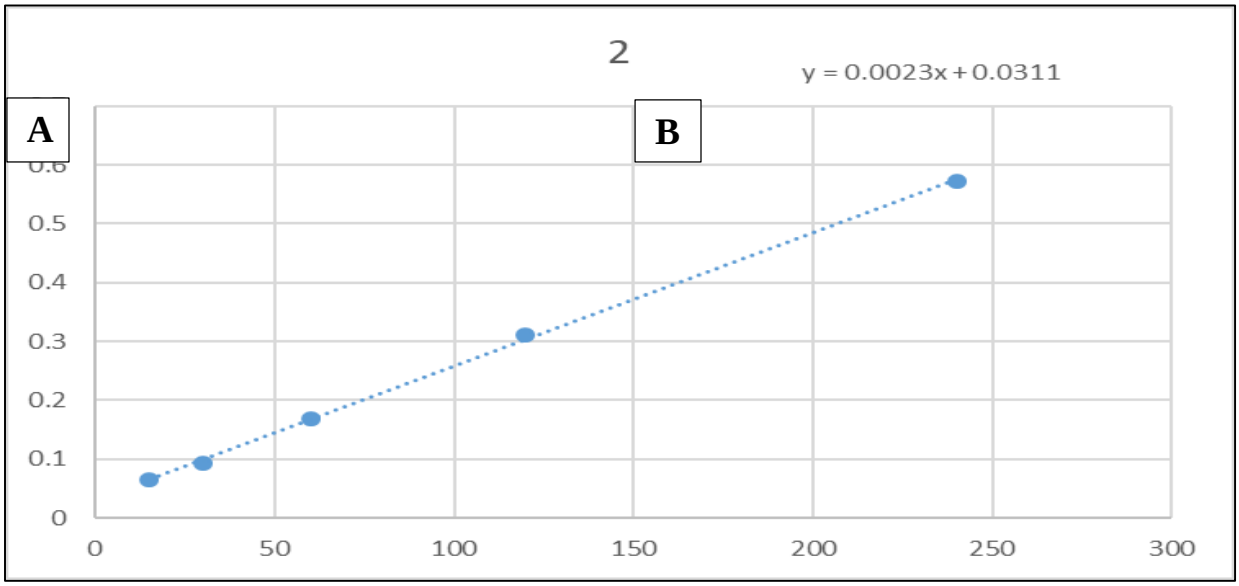


Figure (3.3): standard curve of kallistatinen

(1) Estimation of blood parameters) Appendix (5)

Test procedure:

1. Check the operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
2. Switch the instrument on by pressing the ON/OFF switch, located on the back of the instrument.
3. The instrument performs an initialization phase for the internal electronics.
    a. Please wait.
4. Once the initialization phase is complete, the ABX Micros ES60 OT/CT will automatically run a startup cycle.
5. If the ABX does not automatically run a startup cycle after the initialization phase is completed, press "Startup" button in the "Status" area to initiate a startup cycle.

6. Then, the instrument will perform a blank cycle for a reference blank count (an analysis cycle based on reagents without any blood sample).
7. Check and verify that the reference blank counts do not exceed the following parameter limits: WBC < 0.3, RBC < 0.02, HGB < 0.3, PLT <10 then: Press "OK" button to validate blank results.
8. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.
9. Entering patient ID, sample ID, Patient name, etc.
10. Follow the indications displayed in the "Sample analysis" dialog box to run the analysis.

a. Mix the sample gently and thoroughly.
b. Remove the cap from the sample tube.
c. Place the sample beneath the sampling needle.
d. Raise up the tube so that the sampling needle lowers into the blood and press the manual sample bar.
e. The analysis cycle will begin.

11. When the analysis is completed, the "Sample analysis" dialog box is closed and results are displayed in the "Result display" menu for print out.
12. Dilute the sample if White blood cell counts ≥100,000 /mm3 and platelet counts ≥1,000,000 /mm3 are outside the linearity specifications of the instrument.

Appendix (6): Measuring of AST (alanine aminotransferase), ALT (glutamic-oxaloacetic transaminase), ALP (Alkaline Phosphatase Test).

This test has been formulated according to the standardized method described by IFCC (2002).

Procedure

1. Preincubate working reagent, samples, and controls to reaction temperature.
2. Set the photometer to 0 absorbances with distilled water.
3. Pipette into a cuvette:

Reaction temperature	37ºC	30ºC
Working reagent	1.0mL	1.0mL
Sample or control	50 µL	100 µL

4. Mix gently by inversion. Insert cuvette into the and starter stop watch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after, 1, 2 and 3minutes.
7. Calculate the difference between absorbances.
8. . Calculate the mean of the results to obtain the average change in absorbance per minute (A/min).

Calculation U/L = A/min × 3333 (37ºC).

Appendix(7:Rat liver type facid-bindingnding protein ELISA kit Assay (L-FABP) procedure of Assay

1. Prepare all reagents, standard solutions and samples as instructed.Bring all reagents to room temperature before use.The assay is performed at room temperature.
2. Determine the number of strips required for the assay .Insert the strips in the frames for use.The unused strips should be stored at 2-8 C.
3. Add 50 µl standard to standard well.Note: Donot add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.

4. Add 40 µl sample to sample wells and then add 10µ anti-L-FABP antibody to sample wells, then add 50 µl streptavidin –HRP to sample wells and standard wells (Not blank control well).Mix well .Cover the plate with a sealer .Incubate 60 minutes at 37 C.

5. Remove the sealer and wash the plate 5 times with wash buffer.Soak wells with 300µl wash buffer for 30 seconds to 1 minute for each wash.For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well .Incubate plate covered with a new sealer for 10 minutes at 37 C in the dark.

7. Add 50 µl stop solution to each well, the blue color will change into yellow immediately

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Rat Kallistatin ELISA Kit Appendix (8)

This ELISA kit is referred to as an enzyme-linked immunosorbent assay

(ELISA). The plate has been pre-coated with rat L-FABP antibody. LFABP from the sample binds to the coated antibodies in the wells when it is introduced. The biotinylated rat L-FABP antibody is then added and binds to the sample's L-FABP. After that, streptavidin-HRP is used to bind the biotinylated L-FABP antibody. After incubation, unbound Streptavidin-HRP is eliminated by a washing process. After adding substrate solution, the amount of Rat L-FABP1 is then adjusted based on the color results. The process is halted by adding an acidic stop solution, and after that, absorbance is measured at 450 nm.

The value for kallistatin was estimated according to the manual procedure of the BT LAB kit (chine).

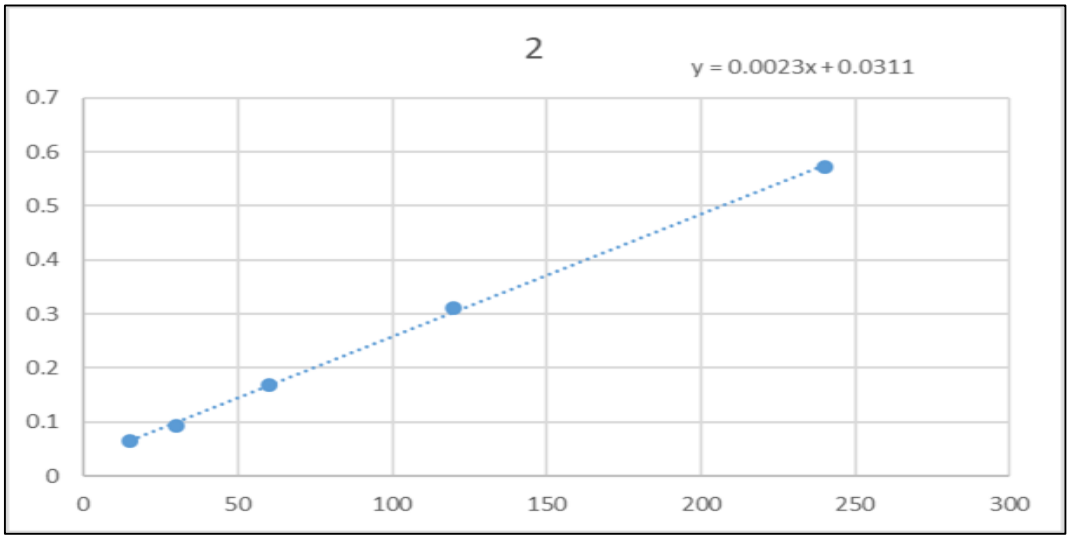


Figure (3.2): the standard curve of kallistatin

Appendix (9) : procedure of Assay Rat Kallistatin ELISA Kit

1. 1.Prepar all reagents,standard solutions and samples as instructed.Bring reagents to room temperature before use.The assay is performed at room temperature.
2. 2.Determine the number of strips required for the assay.Insert the strips in the frames for use.The unused strip should be storred at 2-8 C.
3. 3.Add 50µl standard to standard well.Note:Donnot add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells ad then add 10 µl anti-SERPINA4 antibody to sample wells,then add 50 µl streptavidin-HRP to sample wells and standard wells (Not blank control well).Mix well.Cover the plate with a sealer.Incubate 60 minutes at 37 ºC.
5. Remove the sealer and wash the plate 5 times with wash buffer.Soak wells with 300 µl wash buffer for 30 seconds to 1 minute for each wash.For

automated washing, aspirate or decant erch well and wash 5 times with wash buffer.Blot the plate onto paper towels or other absorbent material.

6. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well.Incubate plate covered with a new sealer for 10 minutes at 37 ºC in the dark.
7. Add 50 µl stop solution to each well, the blue color will change into yellow immediately
8. 8.Determine the optical density (OD value) of each well immediately using reader set to 450 nm within 10 minutes after adding the stop solution.

Principle of Test Appendix) 10)

The kit in question is an enzyme-linked immunosorbent assay (ELISA). The plate has been pre-coated with Rat SERPINA4 antibody. The sample's SERPINA4 binds to the antibodies that have been coated on the wells when it is injected. The biotinylated rat SERPINA4 antibody is then added and binds to the sample's SERPINA4. After that, streptavidin-HRP is added to bind the biotinylated SERPIN4 antibody. Unbound Streptavidin-HRP is removed during a washing step after incubation.

Then, substratum solution is introduced, and color emerges in accordance with the Rat SERPINA4 concentration. By adding an acidic stop solution, the process is stopped, and absorbance is measured at 450 nm.

Appendix (11): Histological processing and staining Processing steps

1. The collected tissue sample was fixed to preserve tissue from degradation (autolysis and bacterial degradation) and to maintain the structure of the tissue byusingd formalin as fixative at a concentration of 10% for 24 hours.
2. Dehydration by removal of water from the tissue is necessary since water is not miscible with paraffin wax that is used in embedding, for removal of water, the tissue is submersed successively in alcohol solutions of increasing concentrations of ethanol (ascending series of 50 ,70,8 ,90,100,100,100% for two hours to each concentration.
3. Clearing since alcohol was not miscible with paraffin wax, it had to be removed from the tissue : this was done by xylene for one ,two then two hours.
4. Infiltration via used a mix of xylene and paraffin wax (melting point 56_58 ºC) then put modules in electric oven temperature 60 ºC for 30 minutes.
5. Transported to molten wax for one hour Embedding in paraffin wax and left at laborator temperature.
6. Sectioning by microtome(5-µm-thick sections from wax-impregnated tissue are obtained), a small block of paraffin wax containing the tissue was prepared and by using the microtome around 5-µm- thick sections from wax-impregnated tissue were obtained, these wax-impregnated sections were floated on warm water (45ºC); this allows the wax to fatten out then sections.

Haematoxylin-Eosin (H and E) staining Technique (APP12)

The sections were stained with Hematoxylin and Eosin according to the method of Bancroft and Gamble (2002) as follows:

1. Deparaffinization: the slides was placed of in xylene for 10 minutes.
2. Hydration : the tissue section was hydrated by passing through gradual descending concentrations of alcohol(100,90,80 and 70%) and water.
3. Nuclear staining : tissue section was stained in haematoxylin –Harris for 3 minutes .
4. Tissue section then washed in running tap water for 5 minutes until section colored blue.
5. Differentiation :Tissue section was dipped in 1% acid alcohol for 5-10 seconds.
6. Slides was stained in 1% eosin for 1 minute.
7. Tissue section was washed in running tap water for 5-10 minutes.
8. Dehydration :the tissue section was hydrated by passing through gradual concentrations of ethanol (70,80,90 and 100 %)for two minutes to each phase.
9. Clearing: Slide was placed in xylene for 2 minutes , and mount with disteren plasticizer xylene (DPX).

Table (4-1) level of some blood parameters in male rats treated with Isotretinoin (20 and 40) mg/kg, in different periods for 30 days.

Groups / Parameters	Control (D.W negative)	Control (Oil) positive	20 (mg/Kg)			40 (mg/Kg)			LSD (0.05)
			24h	48h	72h	24h	48h	72h	
	Mean±S.D								
WBC($\times10^9$/L)	8.17±4.7	8.23± 2.4	13.86 ±4.7	7.87± 1.9	4.95± 1.4	5.61± 0.9	10.03±2.0	7.38± 1.4	3.800
Lym(%)	74.38±8. 8	66.48± 6.4	57.40±8. 1	54.22±6. 4	73.56±11 .1	66.84± 13.4	57.32±6.5	63.85±3 .8	9.203
Mid(%)	2.88± 0.6	4.20± 0.9	5.66±0.9	5.50± 0.6	3.68± 0.4	3.66± 0.3	5.54± 0.5	4.75± 0.3	1.264
Gran(%)	22.75±7. 1	29.32± 5.7	36.94±7. 4	40.28±5. 8	22.74±4. 5	29.50± 7.2	37.14±6.2	31.40±3 .4	8.138
RBCs ($\times10^{12}$/L)	5.80± 0.3	5.74± 0.4	4.43± 0.5	5.58± 0.6	5.10± 1.1	3.73± 0.4	4.96± 0.5	5.52± 0.6	1.049
HGB (g/dL)	15.40±0. 3	13.78± 0.5	10.16±1. 1	11.70±1. 8	13.15±0. 3	9.58± 0.7	13.30±1.2	12.23±2 .7	1.476
HCT(%)	45.20±0. 9	42.23±1.4	31.75±4. 6	31.50±5. 5	32.08±2. 0	26.02± 2.6	32.92±3.3	34.33±1 .4	5.715
MCV (fl)	55.63±4. 0	52.00± 2.3	52.56±2. 5	53.06±1. 4	50.80±3. 3	53.68± 2.6	53.54±3.9	54.25±2 .6	3.160
MCH(Pg)	25.78±2. 2	23.56± 1.6	22.06±0. 9	21.04±1. 0	21.94±1. 4	23.44± 1.5	21.68±1.6	22.275± 1.2	1.578
MCHC(g/dL)	46.25±1. 6	45.23± 1.2	42.00±1. 7	39.66±1. 9	43.20±1. 7	43.64± 1.8	40.46±0.7	41.10±1 .5	1.612
PLT ($\times10^9$/L)	421.25±2 6.1	448.40±3 0.9	249.40± 21.2	584.00±2 7.3	262.20±3 5.8	441.40±3 0.6	494.40±2 9.3	413.50± 23.3	306.082

جمهـــــــــورية العـــــــــراق
وزارة التعليم والعالي والبحث العلمي
جامعـــة بابـــــل / كلية العلوم
قســـــم علـــوم الحياة

دراسة فسلجية ونسجية
لكبد ذكور الجرذان البيض المعاملة بعقار الريتينول (الريتان)

رسالة مقدمة الى

مجلس كلية العلوم – جامعة بابل كجزء من متطلبات نيل درجة الماجستير في العلوم / علوم حياة

من قبل

زينب محمد عباس حسون

بكالوريوس علوم حياة / جامعة بابل / 2019

بإشراف

ا.د. حسين جاسم عبيد الحربي

1444هـ **2022 م**

الخلاصة

هدفت الدراسة الحالية لمعرفة تأثير استخدام مدد وجرعات مختلفة من عقار الايزوتريتونيون الذي يعد العلاج الأكثر فعالية والمتاح حاليا لعلاج حب الشباب حيث يوصى باستخدام حمض الايزوتريتونيون او 1- سيس – ريتنويك كعلاج بديل عن العلاجات السابقة بالمضادات الحيوية أو الأدوية الموضعية. ولدراسة تأثيره على وظيفة ونسيج الكبد بالإضافة إلى تأثيره على بعض معايير الدم، كذلك لمعرفة تأثيره على بعض الدلائل الحيوية التي توضح مدى تضرر نسيج الكبد .

أجريت الدراسة الحالية للمدة من(تشرين الثاني) 2021(إلى حزيران) 2022 (في البيت الحيواني التابع إلى قسم علوم الحياة , جامعة بابل .

أستخدم في هذه الدراسة(40) جرذا ذكرا أبيضا قسمت الى ثمان مجاميع ضمنت كل مجموعة 5 جرذان المعاملة لمدة 30 يوما بالعقار وقسمت عل النحو الآتي :

المجموعة الأولى عوملت بالماء فقط وعدت مجموعة سيطرة سالبة , المجموعة الثانية عوملت بزيت الكانولا الذي هو صنف من بذور اللفت أو الخردل بمقدار 0.6 مل يوميا وعدت سيطرة موجبة ,المجموعة الثالثة عوملت بعقار الايزوتريتونيون 20 ملغم /كغم يوميا , المجموعة الرابعة عوملت بعقار الايزوتريتونيون 20 ملغم / كغم كل 48 ساعة , المجموعة الخامسة عوملت بعقار الايزوتريتونيون) 20 ملغم \كغم كل 72 ساعة ,المجموعة السادسة عوملت بعقار الايزوتريتونيون 40 ملغم \ كغم يوميا , المجموعة السابعة عوملت بعقار الايزوتريتونيون 40 ملغم \ كغم كل 48 ساعة , المجموعة الثامنة عوملت بعقار الايزوتريتونيون40 ملغم \ كغم كل 72 ساعة . بعد ذلك في اليوم 31 تم التضحية بالحيوانات بعد تخديرها بالكلوروفورم وتشريحها تم جمع عينات الدم والكبد لغرض تقدير بعض المعايير الدمية وكذلك الفحص النسجي للكبد . شملت المؤشرات الدمية تقدير العد الكلي لكريات الدم البيض WBC النسبة المئوية لخلايا اللمفوسايت LYM% وكذلك الخلايا المحببة Gran% و التي مثلت الحمضية والعدلة والقعدة وكذلك Mid % ثم قياس العدد الكلي لكريات الدم الحمر RBCs) ومعدل خضاب الدم HGB ومكداس الدم HCTعدد الصفيحات الدموية PLT وشملت ايضا مؤشرات الدم معدل الهيموكلوبين الكريي MCH ومعدل حجم الهيموكلوبين الكريMCVمعدل تركيز

الهيموكلوبين الكريي,MCHC كذلك قياس انزيمات الكبد وشملت ناقلة أمين الألانينALT ، ناقلة أمين الاسباراتاتAST ، الفوسفاتيز القلويALP ،المؤشرات الحيوية لضرر الكبد Kallistatin ، . FABP1

أوضحت النتائج أن العدد الكلي لكريات الدم البيض انخفض معنويا p<0.05 عند المدة الزمنية 72 ساعة عند الجرعة 20 ملغم /كغم , والمدة الزمنية 24 ساعة عند الجرعة40 ملغم /كغم ،كما ان النسبة المئوية للخلايا اللمفاوية انخفضت معنويا p<0.0 5 عند المدتين الزمنيتين 24 و48 ساعة وللجرعتين) 40 و20 ملغم /كغم على التوالي ،إما هيموكلوبين الدم فقد انخفض معنويا p<0.05 عند المدة الزمنية 24 ساعة عند الجرعة 40 ملغم /كغم، القيمة لأنواع من خلايا الدم البيضاء المحببة Mid% أظهرت ارتفاعا معنويا P< 0.05 ضمن الحدود الطبيعية لكيلا الجرعتين ولكل المدد الزمنية، كما أظهرت النتائج انخفاض كريات الدم الحمراء معنويا P< 0.05للمدة الزمنية 24 ساعة عند الجرعة 40ملغم /كغم، بينما أظهرت النتائج مكداس الدم HCT انخفاضا معنويا P< 0.05 لكلا الجرعتين ولكل المدد الزمنية، كما لا توجد اي فروقات معنوية P>0.05 لمتوسط حجم كريه الدم الحمراء MCVالمؤشر MCH معدل كمية الهيموكلوبين الكريي انخفض معنويا P< 0.05 لكلا الجرعتين ولكل المدد الزمنية، أظهرت النتائج بأن تركيز هيموكلوبين الكريي MCHC انخفض معنويا P< 0.05 لكلا الجرعتين ولكل المدد الزمنية ،اما عدد الصفائح الدموية في الدم PLT انخفض معنويا P< 0.05 عند المدتين الزمنيتين72 و24 ساعة عند الجرعة) 20 ملغم/كغم , اظهرت النتائج بأن انزيمات الكبد ALP,AST,ALT ارتفعت معنويp≤0.05 كلا الجرعتين ولكل المدد الزمنية. كما اظهرت النتائج بأن المؤشر الحيوي Kallistainen انخفض معنويا P< 0.05 لكلا الجرعتين ولكل المدد الزمنية وهذا دليل على تضرر نسيج الكبد بمعنى كلما انخفضت القيمة ازداد الضرر النسجي للكبد. اما المؤشر الحيوي FABP1 فقد اظهرت النتائج بانه ارتفع معنويا P< 0.05 لكلا الجرعتين ولكل المدد الزمنية وهذا دليل. علئ انه كلما زادت قيمة المؤشر ازداد الضرر النسجي للكبد .

نستنتج من الدراسة ان عقار الايزوتريتونيون كان له تأثير واضح على معايير الدم ووظيفة ونسيج الكبد.